

BigDye X Terminator™ Purification Kit

USER GUIDE

Catalog Numbers 4376484, 4376485, 4376486, and 4376487

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Revision D



Life Technologies Corporation | 2 Preston Court | Bedford, MA 01730

For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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| Revision | Date | Description |
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| D | 2 June 2020 | <ul style="list-style-type: none">• Updated to the current document template, with associated updates to the warranty, trademarks, and logos.• Corrected component storage temperature.• Updated supported injection configurations, compatible instruments, BDx run modules, robotic pipetting method, and instructions for using the BDx Utility.• Made wording and formatting updates. |
| C | July 2007 | Baseline for this revision history. |

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Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The Applied Biosystems™ BigDye XTerminator™ Purification Kit is a rapid purification method for DNA sequencing reactions that removes unincorporated reaction components, such as salt ions, dye terminators, and dNTPs, to prevent their co-injection with dye-labeled extension products.

Key features:

- Single-plate format eliminates the need for sample transfer.
- Simple purification protocol can be performed in under 40 minutes.
- Stabilizes samples before analysis.
- Compatible with manual or automated workflows.
- Generates high-quality sequencing data.
- Compatible with all terminator-sequencing chemistries, reaction volumes, and template types.

The BigDye XTerminator™ Purification Kit contains BigDye XTerminator™ Solution and SAM™ Solution. Features of these reagents include:

- BigDye XTerminator™ Solution—Captures unincorporated dye terminators and free salts from the post cycle-sequencing reaction.
- SAM™ Solution—Improves reagent performance and stabilizes the sample after purification.

Procedure overview

Traditional purification methods, such as ethanol precipitation, require multiple reagents, liquid transfer, and centrifugation steps. The BigDye XTerminator™ Purification Kit requires the addition of only two reagents, which can be added sequentially or as a premixed solution.

In this procedure, the BigDye XTerminator™ reagents are added to the sequencing products, the reaction plate is vortexed, then briefly centrifuged to collect the unincorporated reaction components at the bottom of the wells. The supernatant, containing the purified dye-labeled extension products, is then directly injected into the instrument using specialized BigDye XTerminator™ (BDx) run modules.

Contents and storage

IMPORTANT! Do not freeze the kit reagents.

Table 1 BigDye XTerminator™ Purification Kit

| Contents | Cat. No. 4376486 (100 reactions) | Cat. No. 4376487 (1,000 reactions) | Cat. No. 4376484 (2,500 reactions) | Cat. No. 4376485 (40,000 reactions) | Storage ^[1] |
|------------------------------|--|--|--|---|------------------------|
| BigDye XTerminator™ Solution | 2 mL | 20 mL | 50 mL | 800 mL | 2–8°C |
| SAM™ Solution | 9 mL | 90 mL | 225 mL | 3,600 mL | 15–30°C |

^[1] See packaging for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 2 Materials required for purification and capillary electrophoresis

| Item | Source |
|--|----------------------------------|
| Instruments | |
| One of the following Applied Biosystems™ genetic analyzers: <ul style="list-style-type: none"> • SeqStudio™ Genetic Analyzer • 3500/3500xL Genetic Analyzer with Data Collection v3 Software or later • 3730/3730xL DNA Analyzer with Data Collection v2 Software or later • 3130/3130xL Genetic Analyzer with Data Collection v3 Software or later See the instrument user guide for parts and consumables needed to perform electrophoresis. | Contact your local sales office. |
| Equipment | |
| Standard laboratory mixer (vortex or equivalent) | MLS |
| Adjustable pipettors | MLS |
| Multichannel pipettor | MLS |
| (Optional) Single or multichannel electronic pipettor | MLS |
| (If you are using heat seal film) ALPS 3000 Automated Microplate Heat Sealer | AB3000 |
| Plate vortexer | See Table 3 on page 7. |

Table 2 Materials required for purification and capillary electrophoresis (continued)

| Item | Source |
|---|---------|
| Centrifuge with swinging bucket (with PCR plate adapter) | MLS |
| Tubes, plates, and accessories | |
| Reagent reservoir | MLS |
| Aerosol-resistant pipette tips | MLS |
| Wide-bore (>1.0 mm) pipette tips | MLS |
| MicroAmp™ Clear Adhesive Film | 4306311 |
| MicroAmp™ Adhesive Film Applicator | 4333183 |
| MicroAmp™ Multi Removal Tool | 4313950 |
| (Optional) Heat Seal Film for Sequencing and Fragment Analysis Sample Plates ^[1] | 4337570 |
| MicroAmp™ Splash-Free 96-Well Base | 4312063 |

^[1] Recommended only if you are using the 3730/3730xl instrument.

Table 3 Recommended plate vortexers and accessories

| Item | Source |
|--|--|
| Thermo Scientific™ Digital Vortex Mixer and accessories | |
| Digital Vortex Mixer, 120 V (US/JP plug) | 88882009 |
| Digital Vortex Mixer, 230 V (EU/UK/CHN plug) | 88882010 |
| Microplate Tray | 88882122 |
| (Optional) 96-well PCR tube rack | Fisher Scientific™ 03-448-20 |
| Elastic bands, size 64 (90 mm × 6 mm) (4 bands) | MLS |
| Digital Vortex-Genie™ 2 and accessories | |
| Digital Vortex-Genie™ 2, 120 V, 60 Hz ^[1] | Scientific Industries™ SI-A536 |
| Digital Vortex-Genie™ 2, 100 V, 50/60 Hz ^[1] | Scientific Industries™ SI-A586 |
| Digital Vortex-Genie™ 2, 230 V, 50 Hz (EU plug) ^[1] | Scientific Industries™ SI-A256 |
| Digital Vortex-Genie™ 2, 230 V, 50 Hz (UK plug) ^[1] | Scientific Industries™ SI-A266 |
| (Optional) Accessories required for other Digital Vortex-Genie™ 2 models: <ul style="list-style-type: none"> Shock-absorbing feet Microplate adapter (includes 2 elastic bands) Replacement elastic bands (2 bands) | Scientific Industries™ : <ul style="list-style-type: none"> 0K-0400-900 SI-0513 0K-0513-900 |

Table 3 Recommended plate vortexers and accessories (continued)

| Item | Source |
|--|------------------------------------|
| (Optional) Recessed platform | Scientific Industries™ 504-0039-00 |
| (If using a recessed platform) Elastic bands (2 bands) | Scientific Industries™ 568-0001-00 |
| IKA™ MS 3 Digital Orbital Shaker and accessories | |
| IKA™ MS 3 Digital Orbital Shaker ^[2] | Fisher Scientific™ NC1476321 |
| Elastic bands, size 64 (90 mm × 6 mm) | MLS |
| Electrical plug adapter ^[3] | MLS |
| Taitec MicroMixer E-36 vortexer and accessories | |
| Taitec MicroMixer E-36 vortexer | Bionexus™ BNE36 |
| MicroAmp™ 96-Well Base | N8010531 |
| Union Scientific™ Vertical Shaker and accessories | |
| Union Scientific™ Vertical Shaker | Union Scientific™ 9816 |
| MicroAmp™ Splash-Free 96-Well Base | 4312063 |
| IKA™ Vortex 3 and accessories^[4] | |
| IKA™ Vortex 3 | IKA™ 0003340001 |
| IKA™ VG 3.3 Universal attachment | IKA™ 0003342400 |
| Elastic bands, size 117B (3 mm × 178 mm) | MLS |
| Eppendorf™ MixMate™ vortexer^[5] | |
| Eppendorf™ MixMate™ vortexer, 120 V, 50/60 Hz | Fisher Scientific™ 21-379-00 |
| Eppendorf™ MixMate™ vortexer, 240 V, 50/60 Hz | MLS |

^[1] Includes all required accessories.

^[2] Includes the MS 3.4 microtiter attachment.

^[3] Required only for use outside of North America.

^[4] Compatible with 96-well plate formats only.

^[5] Compatible with 384-well plate formats only.

Supported configurations for direct injection

Note: If direct injection is not supported for your configuration, the supernatant can be manually transferred to a new plate.

Table 4 Supported instruments, software, and seals for direct injection

| Plate or tray type ^[1] (reaction volume per well) | Seal | Direct injection supported | Run module |
|---|--------------------|----------------------------|------------|
| SeqStudio™ instrument | | | |
| 96-well (10-µL) | Septa | Yes | BDx |
| 96-well (20-µL) | Septa | Yes | BDx |
| 3500/3500xL instrument with Data Collection v3 Software or later | | | |
| 384-well (5-µL) | Septa | Yes | BDx |
| 96-well (10-µL) | Septa | Yes | BDx |
| 96-well (20-µL) | Septa | Yes | BDx |
| 3730/3730x/ instrument with Data Collection v2 Software or later | | | |
| 384-well (5-µL) | Heat seal | Yes | BDx |
| | Septa | No | Standard |
| 96-well (10-µL) | Heat seal or septa | Yes | BDx |
| 96-well (20-µL) | Heat seal or septa | Yes | BDx |
| 3130/3130x/ instrument with Data Collection v3 Software or later | | | |
| 384-well (5-µL) | Septa | No | Standard |
| 96-well (10-µL) | Septa | Yes | BDx |
| 96-well (20-µL) | Septa | Yes | BDx |
| ABI PRISM™ 310 Genetic Analyzer | | | |
| 96-well (10-µL) | Septa | No | Standard |
| 96-well (20-µL) | Septa | No | Standard |

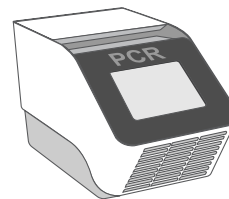
^[1] Depending on the instrument used.

Workflow

Sequencing workflow

Start with cycle sequencing products

See “Guidelines for sequencing reactions” on page 11.



Add the BigDye XTerminator™ reagents to the reaction plate

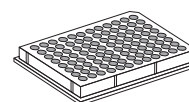
Add the reagents sequentially or as a premixed solution.



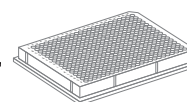
5 m

Note: If you are using a robotic pipetting system, see Appendix D, “Robotic Pipetting method”.

IMPORTANT! Keep the reagents thoroughly mixed throughout the pipetting procedure.



96-well plate



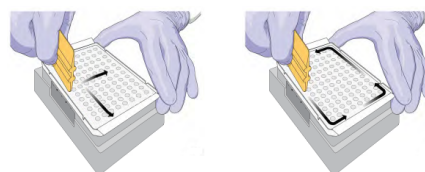
384-well plate

Seal the reaction plate

Apply a MicroAmp™ Clear Adhesive Film to the plate. *(Optional)* If you are using the 3730/3730xl instrument, apply a heat seal using standard heat-sealing techniques.



3 m



Vortex, then centrifuge the reaction plate

Vortex the sealed plate for the specified time, then briefly centrifuge to collect the unincorporated reaction components at the bottom of the wells.



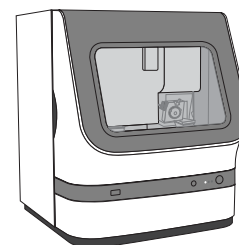
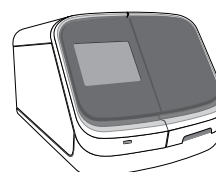
20 m
or
30 m

STOPPING POINT The purified sequencing reactions are stable at room temperature for up to 48 hours. See “Guidelines for storing the reaction plate” on page 19.



Perform capillary electrophoresis

Prepare the plate, then run capillary electrophoresis.





Perform purification, then analyze the samples

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Guidelines for sequencing reactions

- DNA sequencing reactions that are purified with the BigDye XTerminator™ Purification Kit result in high signal strength when analyzed. If needed, decrease the amount of DNA template in the sequencing reactions to keep the fluorescence signals on-scale during analysis.

Note: If the template concentration is decreased, the amount of any template controls must be decreased proportionately.

- Ensure the sequencing reactions meet the minimum volume requirements according to the following table.

If needed, adjust the reaction volume with UltraPure™ DNase/RNase-Free Distilled Water before purification with the kit.

| Plate type | Minimum reaction volume |
|------------|-------------------------|
| 384-well | 5 µL |
| 96-well | 10 µL ^[1] |

^[1] If you are processing >48 samples for a single capillary electrophoresis run, we recommend a minimum reaction volume of 20 µL.

Guidelines for pipetting

The following guidelines apply to single and multichannel pipettors.

-
- **IMPORTANT!** Keep the reagents thoroughly mixed throughout the pipetting procedure.
-

- Keep the reagent caps closed between pipetting steps.
- Use wide-bore tips (with an orifice >1.0 mm) to pipet the BigDye XTerminator™ Solution. Do not use conventional tips.

Note: Wide-bore tips ensure that the correct volume of the slurry is added to the sequencing reactions.

- Use conventional tips to pipet the SAM™ Solution and Premix Solution.
- Ensure the pipette tip is below the surface of the liquid when pipetting the BigDye XTerminator™ Solution.
- **For the Sequential Pipetting method:**
 - Do not aspirate more volume than you can pipette in approximately 1 minute.
 - Dispense the reagents against the side of the well, using the fastest speed setting.
- **For the Premix Pipetting method:**
 - Agitate the Premix Solution before each aspiration.
 - Use an 8- or 12-channel P200 pipette, if available, to add the Premix Solution to the reaction plate.
 - Do not aspirate more Premix Solution than you can pipette in approximately 20–30 seconds.
 - If you are pipetting from a reagent reservoir, gently rock the reservoir back-and-forth 2–3 times to mix, then aspirate the Premix Solution from the bottom of the reservoir.
 - If you are pipetting from a bottle, agitate the Premix Solution using a rocking motion. Do not use a stir bar on a stir plate.

Guidelines for vortexing

- Perform all vortexing steps as described in the procedure.
-

IMPORTANT! Insufficient vortexing or vortexing beyond the specified time can result in low-quality sequencing data.

- Use a recommended vortexer that is appropriate for the number and type of reaction plates that you plan to use (see Table 3 on page 7).

Note: If an alternative vortexer is used, we recommend an instrument with a maximum orbital diameter of 4 mm.

- For set-up and operating instructions specific to your instrument, see Appendix B, “Vortexer set-up”.

Before you begin

- Select the pipetting method that is appropriate for your laboratory.
 - **Sequential Pipetting method**—See “Sequential Pipetting method” on page 13.
 - **Premix Pipetting method**—See “Premix Pipetting method” on page 14.
 - **Robotic Pipetting method**—See Appendix D, “Robotic Pipetting method”.
- Inspect the SAM™ Solution. If precipitates are visible, warm the solution at 37°C, then gently mix to dissolve the precipitates. Cool the solution to room temperature before use.
- Thoroughly mix the SAM™ Solution.
If foam develops after mixing, allow the foam to settle before use.
- If you are using the direct injection method, ensure the appropriate BDX run module is installed and updated on your system (see “BDX run modules” on page 34).

Add the BigDye XTerminator™ reagents to the reaction plate

Sequential Pipetting method

In this procedure, the BigDye XTerminator™ Solution is added to the sequencing reactions followed by addition of the SAM™ Solution.

Sequentially add the reagents to the sequencing reactions

1. Centrifuge the cycle-sequencing reaction plate at 1,000 × *g* for 1 minute.
2. Immediately before pipetting, mix the BigDye XTerminator™ Solution by inversion 10 times or until the solution is homogeneous.
3. Add the following components (in the order indicated) to each well of the reaction plate.

IMPORTANT! Use wide-bore tips to pipet the BigDye XTerminator™ Solution.

IMPORTANT! Remix the BigDye XTerminator™ Solution every minute to prevent phase separation.

| Component | Volume per 5-μL reaction | Volume per 10-μL reaction | Volume per 20-μL reaction |
|------------------------------|--------------------------|---------------------------|---------------------------|
| SAM™ Solution | 22.5 μL | 45 μL | 90 μL |
| BigDye XTerminator™ Solution | 5 μL | 10 μL | 20 μL |

4. Seal the plate according to your sealing method.
 - Using a MicroAmp™ Clear Adhesive Film—Proceed to “Seal the reaction plate” on page 16.
 - Using a heat seal—Apply a heat seal at 160°C for 1.5 seconds, then proceed to “Vortex, then centrifuge the reaction plate” on page 16.

Premix Pipetting method

In this procedure, the BigDye XTerminator™ Solution and SAM™ Solution are combined to form the Premix Solution. The Premix Solution is added to the sequencing reactions in a single step.

Prepare the Premix Solution

Prepare fresh Premix Solution for each processing run.

1. Immediately before pipetting, mix the BigDye XTerminator™ Solution by inversion 10 times or until the solution is homogeneous.
2. Combine the following components (in the order indicated) in an appropriately-sized bottle or reagent reservoir according to one of the following tables.

Calculate the number of wells. Scale the components proportionally based on the volume per well, then add 20% overage.

IMPORTANT! Use wide-bore tips to pipet the BigDye XTerminator™ Solution.

Table 5 384-well plate, 5-µL reaction volume

| Component | Volume | |
|------------------------------|----------------|------------------------|
| | 1 well | 1 plate ^[1] |
| BigDye XTerminator™ Solution | 5 µL | 2,304 µL |
| SAM™ Solution | 22.5 µL | 10,368 µL |
| Total Premix Solution | 27.5 µL | 12,672 µL |

^[1] Includes 20% overage.

Table 6 96-well plate, 10-µL reaction volume

| Component | Volume | |
|------------------------------|--------------|------------------------|
| | 1 well | 1 plate ^[1] |
| BigDye XTerminator™ Solution | 10 µL | 1,152 µL |
| SAM™ Solution | 45 µL | 5,184 µL |
| Total Premix Solution | 55 µL | 6,336 µL |

^[1] Includes 20% overage.

Table 7 96-well plate, 20-µL reaction volume

| Component | Volume | |
|------------------------------|---------------|------------------------|
| | 1 well | 1 plate ^[1] |
| BigDye XTerminator™ Solution | 20 µL | 2,304 µL |
| SAM™ Solution | 90 µL | 10,368 µL |
| Total Premix Solution | 110 µL | 12,672 µL |

^[1] Includes 20% overage.

Add the Premix Solution to the sequencing reactions

IMPORTANT! Keep the Premix Solution thoroughly mixed throughout the pipetting procedure.

1. Centrifuge the cycle-sequencing reaction plate at 1,000 × *g* for 1 minute.
2. Immediately before pipetting, mix the Premix Solution.
 - For Premix Solution in a bottle—Cap the bottle, then mix by inversion 10 times or until the solution is homogeneous.
 - For Premix Solution in a reagent reservoir—Pipet up and down 2–3 times or until the solution is homogeneous.
3. Add the Premix Solution to each well of the reaction plate according to the following table.

IMPORTANT! Agitate the solution before each aspiration.

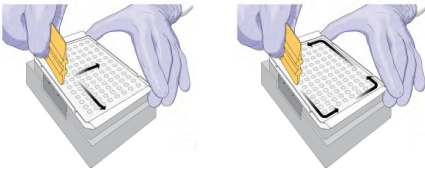
| Plate type (reaction volume per well) | Volume of Premix Solution per well |
|---------------------------------------|------------------------------------|
| 384-well (5-µL) | 27.5 µL |
| 96-well (10-µL) | 55 µL |
| 96-well (20-µL) | 110 µL |

Discard any remaining Premix Solution after use.

4. Seal the plate according to your sealing method.
 - Using a MicroAmp™ Clear Adhesive Film—Proceed to “Seal the reaction plate” on page 16.
 - Using a heat seal—Apply a heat seal at 160°C for 1.5 seconds, then proceed to “Vortex, then centrifuge the reaction plate” on page 16.

Seal the reaction plate

IMPORTANT! Apply firm pressure to the adhesive film during application to ensure a tight, leak-proof seal during vortexing.

1. Place the reaction plate on a MicroAmp™ Splash-Free 96-Well Base, then wipe off any liquid on the surface of the plate.
2. Remove a single adhesive film from the box. While holding the film backing-side up, bend both tabs upward.
3. In one swift movement, peel back the white backing from the center sealing surface. Do not touch the center sealing surface.
4. While holding the film by the tabs, lower the film onto the reaction plate (adhesive side facing the plate). Make sure that the film completely covers all wells of the reaction plate.
5. While applying firm downward pressure, move the applicator slowly across the film, both horizontally and vertically.
6. Repeat step 5 five times. While applying pressure, run the edge of the applicator along all four sides of the outer border of the film.
7. Inspect the reaction plate to confirm that all wells are sealed. The plate is properly sealed when an imprint of each well is visible on the surface of the film.
8. Proceed to “Vortex, then centrifuge the reaction plate” on page 16.

Vortex, then centrifuge the reaction plate

IMPORTANT! This kit is optimized for use with the vortexers specified in Table 3 on page 7.

1. Inspect the reaction plate to confirm that all edges, corners, and spaces between the rows and columns of the wells are completely sealed.
2. Firmly attach the plate to the vortexer.
For set-up and operating instructions specific to your instrument, see Appendix B, “Vortexer set-up”.

- Vortex the plate for 20 minutes (96-well plate) or 30 minutes (384-well plate), using the settings indicated.

IMPORTANT! Do not vortex beyond the specified time.

| Vortexer | Plate type | Speed or setting |
|--|---------------------|--------------------------|
| Thermo Scientific™ Digital Vortex Mixer | 96-well | 1,800 rpm |
| | 384-well | 2,000 rpm |
| Digital Vortex-Genie™ 2 | 96-well | 1,800 rpm |
| | 384-well | 2,000 rpm |
| Eppendorf™ MixMate™ | 96-well | 1,800 rpm |
| | 384-well | 2,600 rpm |
| IKA™ MS 3 Digital Orbital Shaker | 96-well or 384-well | 1,800 rpm (Mode B) |
| IKA™ Vortex 3 | 96-well or 384-well | Setting 5 ^[1] |
| Taitec MicroMixer E-36 | 96-well or 384-well | Maximum |
| Union Scientific™ Vertical Shaker ^[2] | 96-well or 384-well | Setting 100 |

^[1] Use the maximum setting that does not cause the vortexer to become unstable.

^[2] If needed, add additional plates to meet the mass requirements.

- Centrifuge the plate at 1,000 × *g* in a swinging-bucket centrifuge for 2 minutes.
- Immediately proceed to capillary electrophoresis.

STOPPING POINT If you cannot run capillary electrophoresis immediately, store the reaction plate as described (see “Guidelines for storing the reaction plate” on page 19).

Perform capillary electrophoresis

See the appropriate instrument user guide for detailed instructions about preparing the reaction plate and setting up an electrophoresis run.

Prepare the reaction plate for capillary electrophoresis

1. Prepare the reaction plate according to the following table.

IMPORTANT! Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents.

| Instrument | Action |
|----------------------|--|
| SeqStudio™ | For 96-well plates—Remove the adhesive film, then place a septum on the plate. |
| 3500/3500xL | For 96- or 384-well plates—Remove the adhesive film, then place a septum on the plate. |
| 3730/3730xI | For plates sealed with heat seal film—Proceed with the sealed plate. |
| | For plates sealed with adhesive film—Prepare the plate according to the plate type. <ul style="list-style-type: none"> • For 96-well plates—Remove the adhesive film, then place a septum on the plate. • For 384-well plates—Remove the adhesive film, then perform one of the following actions. <ul style="list-style-type: none"> – Apply a heat seal to the plate. – Transfer 10 µL of the supernatant to a new plate, then place a septum on the plate. |
| 3130/3130xI | • For 96-well plates—Remove the adhesive film, then place a septum on the plate. |
| | • For 384-well plates—Remove the adhesive film, transfer 10 µL of the supernatant to a new plate, then place a septum on the plate. |
| 310 Genetic Analyzer | For 96-well trays—Remove the adhesive film, transfer 10 µL of the supernatant to a new plate, then place a septum on the plate. |

2. Proceed to run capillary electrophoresis.

Run capillary electrophoresis

1. Load the reaction plate in the instrument.
2. Set up an instrument run using the appropriate conditions for your instrument.
 - If you are using the direct injection method, use a BDx run module specified in “BDx run modules” on page 34.
 - If the supernatant was transferred to a new plate after purification, see your instrument user guide for the appropriate run module (see “Related documentation” on page 43).
3. Start the electrophoresis run.

(Optional) Store reaction plates that have been at room temperature <48 hours as described (see “Guidelines for storing the reaction plate” on page 19).

Guidelines for storing the reaction plate

Sequencing reactions purified with the BigDye XTerminator™ Purification Kit are stable at room temperature for up to 48 hours.

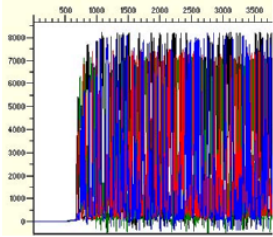
Store reaction plates covered with adhesive film, septa, or heat seal film at room temperature for up to 48 hours.

Note: After removing the reaction plate from storage, centrifuge the plate at $1,000 \times g$ in a swinging-bucket rotor for 2 minutes, then proceed to run capillary electrophoresis.



Troubleshooting

| Observation | Possible cause | Recommended action |
|--|--|--|
| Dye artifacts (blobs) at the beginning of the electropherogram | Incomplete mixing occurred during purification. | <p>Use a recommended vortexer with the appropriate accessories (see Table 3 on page 7).</p> <p>Verify that the vortexer is set up correctly (see Appendix B, “Vortexer set-up”).</p> <p>Follow the vortexing guidelines and procedure as described.</p> <ul style="list-style-type: none"> • See “Guidelines for vortexing” on page 12. • See “Vortex, then centrifuge the reaction plate” on page 16. |
| | The incorrect volume of BigDye XTerminator™ Solution and/or SAM™ Solution was added to the reaction plate. | <p>Add 5 µL of the BigDye XTerminator™ Solution to each sample, seal the plate according to your sealing method, then repeat the vortexing and centrifugation steps (see “Vortex, then centrifuge the reaction plate” on page 16).</p> <p>Ensure the BigDye XTerminator™ Solution and SAM™ Solution are thoroughly mixed throughout the pipetting procedure.</p> <p>Ensure the volume of BigDye XTerminator™ Solution used is equivalent to the starting reaction volume.</p> <p>Ensure the reagents are added at a 4.5:1 (v/v) ratio, SAM™ Solution to BigDye XTerminator™ Solution.</p> <p>Follow “Guidelines for pipetting” on page 12.</p> |
| | Precipitates were present in the SAM™ Solution. | <p>Ensure the SAM™ Solution is stored at room temperature.</p> <p>Warm the solution at 37°C, then gently mix to dissolve the precipitates. Cool the solution to room temperature before use.</p> |
| | The plate seal leaked. | See “The wells of the reaction plate are leaking” on page 25. |
| | Insufficient DNA template was used in the reaction. | Increase the DNA template concentration. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| <p>Off-scale signal on the electropherogram</p>  | <p>The DNA template concentration was too high.</p> | <p>Immediately re-inject the samples. Second injections frequently result in acceptable data quality.</p> |
| | | <p>Decrease the DNA template concentration.</p> |
| | | <p>Decrease the injection time.</p> |
| <p>Weak signal on the electropherogram</p> | <p>Insufficient DNA template was used in the reaction.</p> | <p>Increase the DNA template concentration.</p> |
| | <p>The sequencing reaction failed.</p> | <p>Increase the injection time.</p> |
| | <p>The injection time was too short.</p> | <p>Repeat the sequencing reaction.</p> |
| | <p>An incorrect run module was used.</p> | <p>Increase the injection time.</p> <ul style="list-style-type: none"> • If you are using the direct injection method, ensure that the appropriate BDx run module was used (see “BDx run modules” on page 34). • If the supernatant is transferred to a new plate after purification, see your instrument user guide for the appropriate run module (see “Related documentation” on page 43). |
| | <p>The BigDye XTerminator™ run module offset was not updated after autosampler recalibration.</p> | <p>If you are using a 3130/3130x/ or 3730/3730x/ instrument with Data Collection v4 Software or earlier, run the Update BDx Utility to update the run module offset (see “Update BDx run modules” on page 37).</p> |
| | <p>Incomplete mixing occurred during purification.</p> | <p>Use a recommended vortexer with the appropriate accessories (see Table 3 on page 7).</p> |
| | | <p>Verify that the vortexer is set up correctly (see Appendix B, “Vortexer set-up”).</p> |
| <p>Follow the vortexing guidelines and procedure as described.</p> <ul style="list-style-type: none"> • See “Guidelines for vortexing” on page 12. • See “Vortex, then centrifuge the reaction plate” on page 16. | | |



| Observation | Possible cause | Recommended action |
|---|---|---|
| Weak signal on the electropherogram (continued) | The incorrect volume of BigDye XTerminator™ Solution and/or SAM™ Solution was added to the reaction plate. | Add 5 µL of the BigDye XTerminator™ Solution to each sample, seal the plate according to your sealing method, then repeat the vortexing and centrifugation steps (see “Vortex, then centrifuge the reaction plate” on page 16). |
| | | Ensure the BigDye XTerminator™ Solution and SAM™ Solution are thoroughly mixed throughout the pipetting procedure. |
| | | Ensure the volume of BigDye XTerminator™ Solution used is equivalent to the starting reaction volume. |
| | | Ensure the reagents are added at a 4.5:1 (v/v) ratio, SAM™ Solution to BigDye XTerminator™ Solution. |
| | | Follow “Guidelines for pipetting” on page 12. |
| | The plate seal leaked. | See “The wells of the reaction plate are leaking” on page 25. |
| | One of the components of the capillary electrophoresis system affected the analysis. Possible issues can include: use of an old array or expired consumables, or lack of regular maintenance. | Troubleshoot the capillary electrophoresis system. See the appropriate instrument user guide for additional information (see “Related documentation” on page 43). |
| | The BigDye XTerminator™ Solution was stored improperly. | Do not store the BigDye XTerminator™ Solution above 25°C. |
| | Hi-Di™ Formamide was used or the samples were heat-denatured. | Follow the procedure as described. Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents. |
| Signal starts later than expected (samples migrate slowly) | The DNA template concentration was too high, or a high-molecular-weight DNA template was used in the sequencing reaction. | Immediately re-inject the samples. Second injections frequently result in acceptable data quality. |
| | | Decrease the DNA template concentration. |
| | | Decrease the injection time. |
| Poor peak resolution or short read lengths | The DNA template concentration was too high, or a high-molecular-weight DNA template was used in the sequencing reaction. | Immediately re-inject the samples. Second injections frequently result in acceptable data quality. |
| | | Decrease the DNA template concentration. |
| | | Decrease the injection time. |
| | The purified extension products degraded. | Prepare fresh samples. Store the sealed plates as described (see “Guidelines for storing the reaction plate” on page 19). |
| | The reaction plate was improperly sealed before storage. | See “The wells of the reaction plate are leaking” on page 25. |



| Observation | Possible cause | Recommended action |
|---|---|---|
| Poor peak resolution or short read lengths (continued) | Less than the recommended amount of SAM™ Solution was added to the reaction plate. | Use a 4.5:1 (v/v) ratio of SAM™ Solution to BigDye XTerminator™ Solution. |
| | An old or improperly sealed septa was used. | Use a new septa. |
| | The sequencing signal was weak. | See “Weak signal on the electropherogram” on page 21. |
| | One of the components of the capillary electrophoresis system affected the analysis. Possible issues can include: use of an old array or expired consumables, or lack of regular maintenance. | Troubleshoot the capillary electrophoresis system. See the appropriate instrument user guide for additional information (see “Related documentation” on page 43). |
| No signal from small extension products | The reaction plate was vortexed beyond the recommended time. | Follow the vortexing procedure as described (see “Vortex, then centrifuge the reaction plate” on page 16). |
| | The reaction plate was stored improperly. | Prepare fresh samples. Store the sealed plates as described (see “Guidelines for storing the reaction plate” on page 19). |
| | The BigDye XTerminator™ reagents were degraded. | Check the expiration date of the reagents. Repeat the purification procedure with new reagents. Follow the correct handling and storage conditions. |
| No signal on the electropherogram | An incorrect run module was used. | <ul style="list-style-type: none"> If you are using the direct injection method, ensure that the appropriate BDx run module was used (see “BDx run modules” on page 34). If the supernatant is transferred to a new plate after purification, see your instrument user guide for the appropriate run module (see “Related documentation” on page 43). |



| Observation | Possible cause | Recommended action |
|---|---|---|
| No signal on the electropherogram (continued) | The incorrect volume of BigDye XTerminator™ Solution and/or SAM™ Solution was added to the reaction plate. | Add 5 µL of the BigDye XTerminator™ Solution to each sample, seal the plate according to your sealing method, then repeat the vortexing and centrifugation steps (see “Vortex, then centrifuge the reaction plate” on page 16). |
| | | Ensure the BigDye XTerminator™ Solution and SAM™ Solution are thoroughly mixed throughout the pipetting procedure. |
| | | Ensure the volume of BigDye XTerminator™ Solution used is equivalent to the starting reaction volume. |
| | | Ensure the reagents are added at a 4.5:1 (v/v) ratio, SAM™ Solution to BigDye XTerminator™ Solution. |
| | | Follow “Guidelines for pipetting” on page 12. |
| | The BigDye XTerminator™ run module offset was not updated after autosampler recalibration. | If you are using a 3130/3130x/ or 3730/3730x/ instrument with Data Collection v4 Software or earlier, run the Update BDx Utility to update the run module offset (see “Update BDx run modules” on page 37). |
| | The plate seal leaked. | See “The wells of the reaction plate are leaking” on page 25. |
| | Sample migration was severely delayed. | See “Signal starts later than expected (samples migrate slowly)” on page 22. |
| The sequencing reaction failed. | Repeat the sequencing reaction. | |
| One of the components of the capillary electrophoresis system affected the analysis. Possible issues can include: use of an old array or expired consumables, or lack of regular maintenance. | Troubleshoot the capillary electrophoresis system. See the appropriate instrument user guide for additional information (see “Related documentation” on page 43). | |
| Hi-Di™ Formamide was used or the samples were heat-denatured. | Follow the procedure as described. Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents. | |
| The SAM™ Solution appears cloudy | Precipitates formed in the SAM™ Solution | Inspect the SAM™ Solution upon receipt, then store at room temperature. |
| | | Warm the solution at 37°C, then gently mix to dissolve the precipitates. Cool the solution to room temperature before use. |



| Observation | Possible cause | Recommended action |
|--|---|--|
| The wells of the reaction plate are leaking | The plate was sealed improperly. | <ul style="list-style-type: none"> For a MicroAmp™ Clear Adhesive Film—Use a MicroAmp™ Adhesive Film Applicator to apply firm downward pressure to all edges, corners, and spaces between the rows and columns of the wells. The plate is properly sealed when an imprint of each well is visible on the surface of the film. For a heat seal—Increase the time and temperature for the heat-sealing step. |
| Non-uniform results for a large number of samples | Incomplete mixing occurred during purification. | Use a recommended vortexer with the appropriate accessories (see Table 3 on page 7). |
| | | Verify that the vortexer is set up correctly (see Appendix B, “Vortexer set-up”). |
| | | Follow the vortexing guidelines and procedure as described. <ul style="list-style-type: none"> See “Guidelines for vortexing” on page 12. See “Vortex, then centrifuge the reaction plate” on page 16. |
| | The BigDye XTerminator™ Solution was dispensed incorrectly. | Follow “Guidelines for pipetting” on page 12. |
| The BigDye XTerminator™ reagents are frozen | The reagents were stored improperly. | Thaw the reagents in a refrigerator before use. Note: The reagents are expected to perform to specification following only one freeze-thaw cycle. |
| | | Follow the correct handling and storage conditions. |
| The BigDye XTerminator™ Solution is too viscous to pipette | The reagent was pipetted and/or mixed improperly. | Use a wide-bore tip (with an orifice >1.0 mm) to pipet the BigDye XTerminator™ Solution. |
| | | Follow “Guidelines for pipetting” on page 12. |
| | The reagent evaporated. | Use fresh reagent. Ensure the reagent bottle is properly sealed before storage. |
| | | Keep the reagent caps closed (to prevent evaporation) between pipetting steps. |



Vortexer set-up

| | |
|--|----|
| ■ Set up the Thermo Scientific™ Digital Vortex Mixer | 26 |
| ■ Set up the Digital Vortex-Genie™ 2 vortexer | 28 |
| ■ Set up the Union Scientific™ Vertical Shaker | 30 |
| ■ Set up the IKA™ MS 3 Digital Orbital Shaker | 31 |
| ■ Set up the Taitec MicroMixer E-36 vortexer | 32 |

Set up the Thermo Scientific™ Digital Vortex Mixer

The Microplate Tray attachment (Cat. No. 88882122) is required for 96- and 384-well plates (see Figure 1).

1. Install the Microplate Tray attachment—Follow the instructions of the manufacturer.

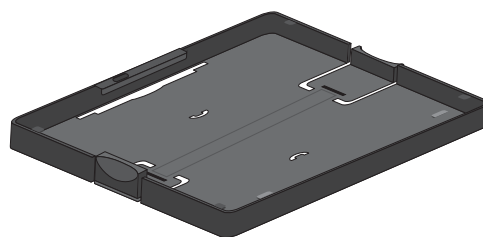


Figure 1 Microplate Tray attachment

2. (Optional) If you are using a 96-well plate, place the sealed plate on a 96-well PCR tube rack (see Figure 2).

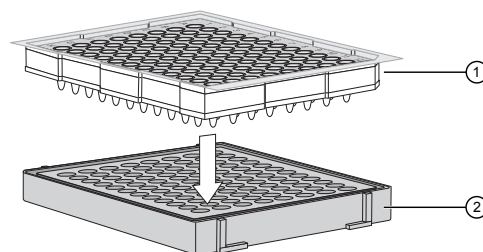


Figure 2 96-well plate/rack assembly

- ① 96-well plate
- ② 96-well PCR tube rack (Fisher Scientific™ 03-448-20)

3. Fasten the plate to the Microplate Tray attachment.

IMPORTANT! Be careful not to disturb the plate sealing film. If damage occurs, remove the seal and replace with a new seal.

- For 96-well plates—Fasten both ends of the plate (or plate/rack assembly) to the attachment using elastic bands (see Figure 3).
- For 384-well plates—Slightly tilt the plate lengthwise, then insert the front and rear of the plate under the tabs (see Figure 4).



Figure 3 Elastic band positions for 96-well plates

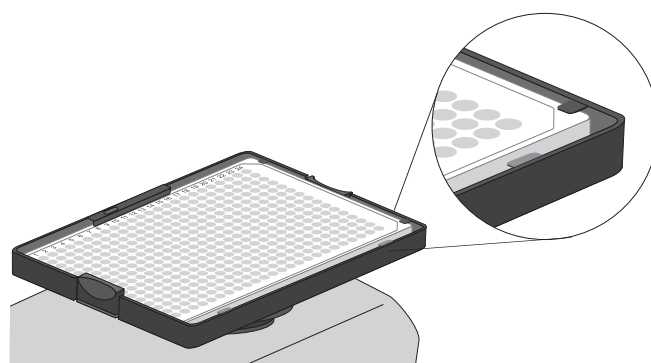


Figure 4 384-well plate inserted into the attachment (showing the tabs securing the plate)

Set up the Digital Vortex-Genie™ 2 vortexer

For a list of compatible accessories, see Table 3 on page 7.

1. (If needed) Attach the following accessories.
 - Replace the stock rubber feet with shock-absorbing feet—Flip the vortexer over. Use a 1/4-inch hex driver to remove the stock feet and attach the shock-absorbing feet.
 - Attach the Microplate Adapter for Applied Biosystems™—Follow the instructions of the manufacturer.

Note: During the installation, be sure to push from the center to avoid bending the adapter.

2. Fasten the plate to the platform.

IMPORTANT! Be careful not to disturb the plate sealing film. If damage occurs, remove the seal and replace with a new seal.

- If you are using the Microplate Adapter for Applied Biosystems™—Fasten the plate to the adapter using elastic bands (see Figure 5).

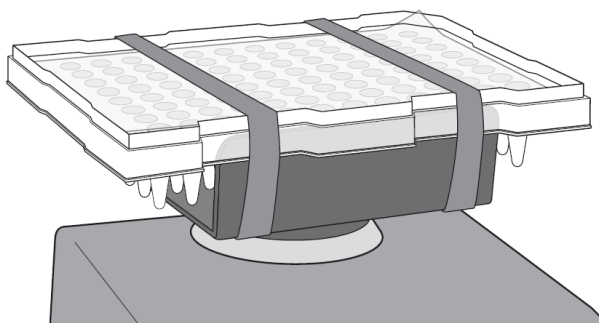


Figure 5 Microplate Adapter for Applied Biosystems™: Elastic band positions for 96- and 384-well plates

- If you are using the recessed platform adapter—Position an elastic band as indicated, according to the plate type.
 - For 96-well plates—See Figure 6.
 - For 384-well plates—See Figure 7.

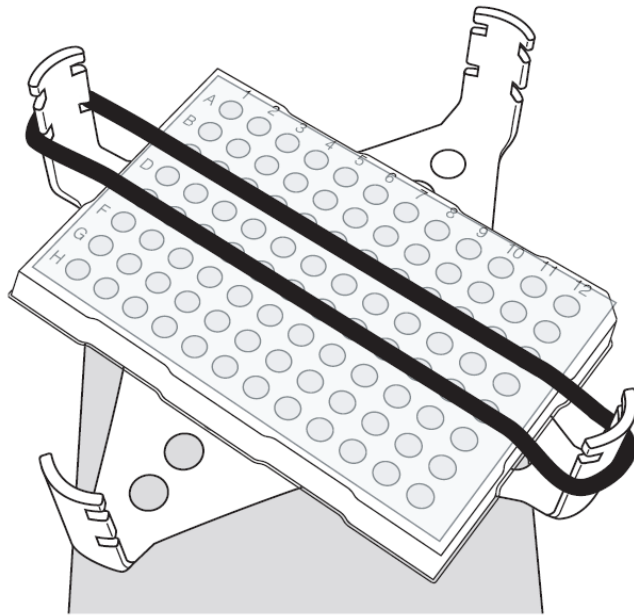


Figure 6 Recessed platform adapter: Elastic band position for 96-well plates

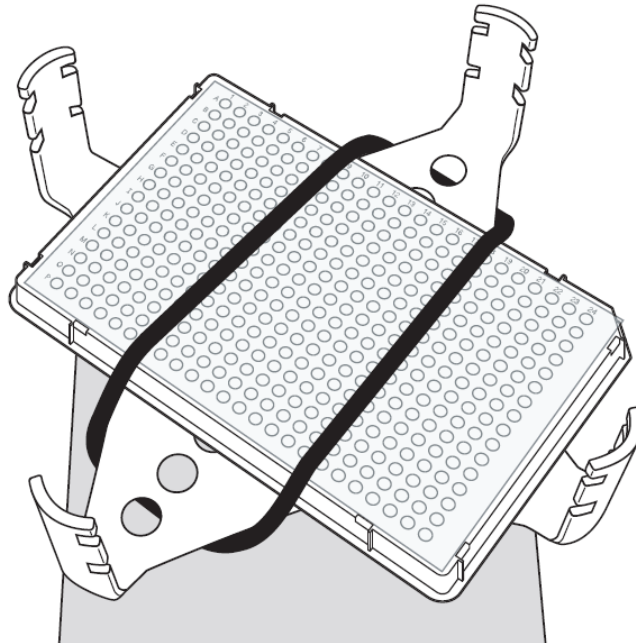


Figure 7 Recessed platform adapter: Elastic band position for 384-well plates



Set up the Union Scientific™ Vertical Shaker

The Union Scientific™ Vertical Shaker requires a sample mass range of 450–2,500 g.

1. Calculate the total mass of the reaction plate(s), reagents, and base(s) using the following table. If needed, include additional empty plates and/or bases to total >450 g.

| Starting reaction volume | Item | Mass |
|--------------------------|---|-------------|
| 5-μL | (Empty) MicroAmp™ Optical 384-Well Reaction Plate | 18 g |
| | Reagents (per well) | 0.033 g |
| | Reagents (per plate) | 12 g |
| | Total mass of reaction plate (+ reagents) | 30 g |
| 10-μL | (Empty) MicroAmp™ Optical 96-Well Reaction Plate | 22 g |
| | Reagents (per well) | 0.065 g |
| | Reagents (per plate) | 6 g |
| | Total mass of reaction plate (+ reagents) | 28 g |
| 20-μL | (Empty) MicroAmp™ Optical 96-Well Reaction Plate | 22 g |
| | Reagents (per well) | 0.130 g |
| | Reagents (per plate) | 12 g |
| | Total mass of reaction plate (+ reagents) | 34 g |
| 10-μL or 20-μL | MicroAmp™ Splash-Free 96-Well Base ^[1] | 68 g |

^[1] Required for 96-well plates only.

Example calculation for three 96-well plates with 10-μL reaction volumes:

- Sum of the plates, reagents, and bases: $(3 \times 68 \text{ g}) + (3 \times 28 \text{ g}) = 288 \text{ g}$
- Additional mass required for operation: $450 \text{ g} - 288 \text{ g} = 162 \text{ g}$
- Possible plate or base combination that totals $\geq 162 \text{ g}$: 3 empty bases ($3 \times 68 = 204 \text{ g}$)

2. Fasten the plate/base assembly to the platform—Follow the instructions of the manufacturer.

IMPORTANT! Be careful not to disturb the plate sealing film. If damage occurs, remove the seal and replace with a new seal.

Set up the IKA™ MS 3 Digital Orbital Shaker

Install the microtiter attachment, then fasten the plate to the attachment

The IKA™ MS 3.4 microtiter attachment is required for both 96- and 384-well plates (see Figure 8).

1. Install the microtiter attachment—
Follow the instructions of the manufacturer.
2. Fasten the plate to the attachment.

IMPORTANT! Be careful not to disturb the plate sealing film. If damage occurs, remove the seal and replace with a new seal.

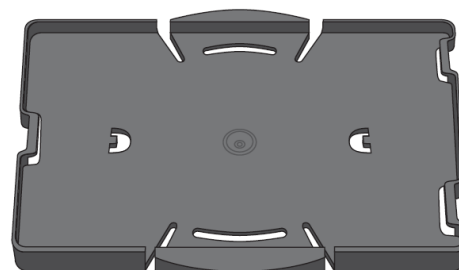


Figure 8 MS 3.4 microtiter attachment

- For 96-well plates—Fasten both ends of the plate to the attachment using elastic bands (see Figure 9).
- For 384-well plates—Slightly tilt the plate lengthwise, then insert the front and rear of the plate under the tabs (see Figure 10).

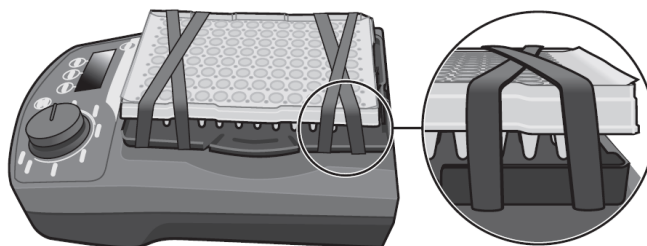


Figure 9 Elastic band positions for 96-well plates

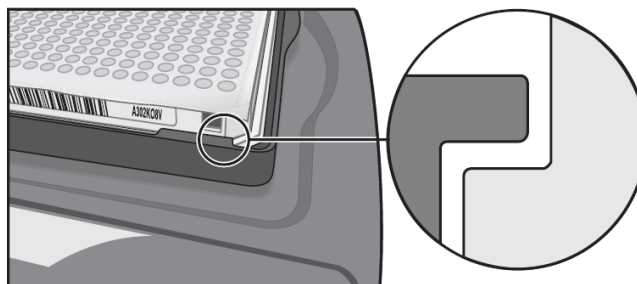


Figure 10 384-well plate inserted into the attachment (showing the tabs securing the plate)

Set the vortexer mode

The IKA™ MS 3 Digital Orbital Shaker has two speed settings: Mode A (low-speed) and Mode B (high-speed). Use Mode B for purification with the BigDye XTerminator™ Purification Kit.

1. Power on the vortexer.
The default setting is Mode A.
2. Set the vortexer to Mode B—Hold down the **Start/Stop** button, then press **Power** (see Figure 11).
Confirm the setting changes to Mode B.

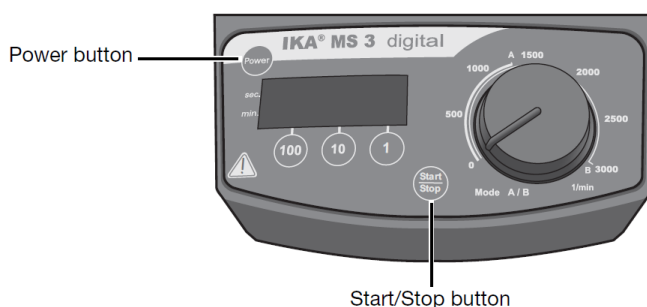


Figure 11 Front panel of the vortexer

Set up the Taitec MicroMixer E-36 vortexer

1. Fasten the plate to the platform.

IMPORTANT! Be careful not to disturb the plate sealing film. If damage occurs, remove the seal and replace with a new seal.

- For 96-well plates—Place the plate on a MicroAmp™ 96-Well Base, then clamp the plate/base assembly to the platform using the white levers.
- For 384-well plates—Place the plate directly on the platform, then clamp the plate to the platform using the white levers.

2. Set the **RANGE** to **HIGH SPEED**.
3. Turn the **SPEED** dial to the maximum speed setting.

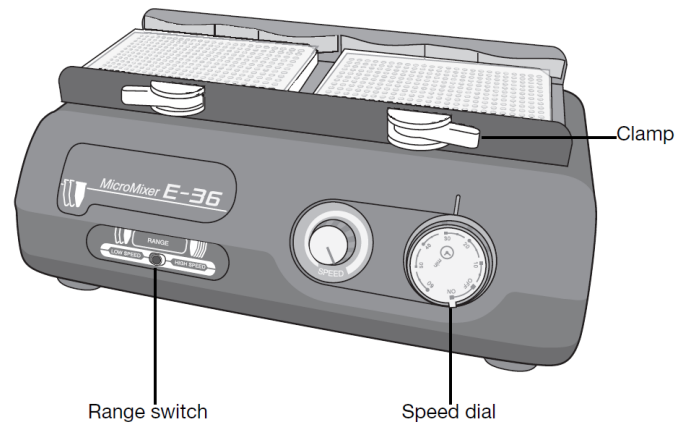


Figure 12 Control panel



BDx run modules

- Overview 34
- BDx run modules 34
- Install BDx run modules (for 3130/3130xl and 3730/3730xl instruments with Data Collection v4 Software or earlier) 36
- Update BDx run modules 37
- Uninstall BDx run modules 38

Overview

BDx run modules adjust the injection height of the instrument to allow direct injection of the sample supernatant following purification with the BigDye XTerminator™ Purification Kit. In addition, BDx run module injection times and voltages are optimized for the high signals obtained from samples purified with the kit.

The BDx Utility installs and calibrates BDx run modules for the Data Collection Software of the instrument. Run the BDx Utility after Data Collection Software installation and every time the instrument autosampler is recalibrated.

Note: The BDx Utility is included in the Data Collection v5 Software installation. No separate download or installation is necessary.

IMPORTANT! Do not use a BDx run module if the supernatant is transferred to a new plate after purification. See the instrument user guide for the appropriate standard run module.

BDx run modules

Determine the appropriate BDx run module for your instrument according to the following tables.

Table 8 BDx run modules for the SeqStudio™ instrument

| Capillary length | Polymer | BDx run module name |
|------------------|----------------------------|---------------------|
| N/A | POP-1™ (universal polymer) | ShortSeq_BDX |
| | | MediumSeq_BDX |
| | | LongSeq_BDX |



Table 9 BDx run modules for the 3500/3500xL instrument

| Capillary length | Polymer | BDx run module name |
|------------------|---------|------------------------|
| 36-cm | POP-7™ | BDxFastSeq36_POP7 |
| | POP-4™ | BDxRapidSeq36_POP4 |
| | POP-6™ | BDxRapidSeq36_POP6 |
| | POP-7™ | BDxRapidSeq36_POP7 |
| 50-cm | POP-6™ | BDxFastSeq50_POP6 |
| | POP-7™ | BDxFastSeq50_POP7 |
| | POP-6™ | BDxRapidSeq50_POP6 |
| | POP-7™ | BDxRapidSeq50_POP7 |
| | POP-7™ | BDxShortReadSeq50_POP7 |
| | POP-6™ | BDxStdSeq50_POP6 |
| | POP-7™ | BDxStdSeq50_POP7 |

Table 10 BDx run modules for the 3730/3730xL instrument

| Capillary length | Polymer | BDx run module name |
|------------------|---------|---------------------|
| 36-cm | POP-7™ | BDx_StdSeq36_POP7 |
| | | BDx_RapidSeq36_POP7 |
| 50-cm | POP-7™ | BDx_XLRSeq50_POP7 |
| | | BDx_LongSeq50_POP7 |
| | | BDx_FastSeq50_POP7 |

Table 11 BDx run modules for the 3130/3130xL instrument

| Capillary length | Polymer | BDx run module name |
|------------------|---------|---------------------|
| 36-cm | POP-7™ | BDx_RapidSeq36_POP7 |
| | POP-6™ | BDx_RapidSeq36_POP6 |
| | POP-7™ | BDx_UltraSeq36_POP7 |
| | POP-4™ | BDx_UltraSeq36_POP4 |
| 50-cm | POP-7™ | BDx_StdSeq50_POP7 |
| | POP-6™ | BDx_StdSeq50_POP6 |
| | POP-4™ | BDx_StdSeq50_POP4 |



Table 11 BDx run modules for the 3130/3130xl instrument (continued)

| Capillary length | Polymer | BDx run module name |
|------------------|---------|---------------------|
| 50-cm | POP-7™ | BDx_FastSeq50_POP7 |
| 80-cm | POP-7™ | BDx_LongSeq80_POP7 |
| | POP-4™ | BDx_LongSeq80_POP4 |

Install BDx run modules (for 3130/3130xl and 3730/3730xl instruments with Data Collection v4 Software or earlier)

Download, then install the BDx Utility

The BDx Utility is included in the Data Collection v5 Software installation. No separate download or installation is necessary.

1. Power on the instrument and the computer with your Data Collection Software.
2. Exit the Data Collection Software if it is running.
3. Go to thermofisher.com/us/en/home/technical-resources/software-downloads/bigdye-xterminator-purification-kit.html.
4. Click **Download**.
5. Double-click the **bdx1.0.1updater.exe** file.
6. Follow the prompts in the **Support Files Installer** wizard.
 - Data Collection v3 Software—The run modules are installed and calibrated based on the instrument's autosampler calibration.
 - Data Collection v3.1, v3.1.x, or v4 Software on 3730/3730xl instruments—The run modules are installed and calibrated based on the instrument's autosampler calibration.
 - Data Collection v3.1, v3.1.x, or v4 Software on 3130/3130xl instruments—Proceed to “Install, then calibrate BDx run modules (for 3130/3130xl instruments only)” on page 37.



Install, then calibrate BDx run modules (for 3130/3130x/ instruments only)

Follow this procedure if you are using the 3130/3130x/ instrument with Data Collection v3.1, v3.1.x, or v4 Software.

In this procedure, the BDx Utility is uninstalled, then reinstalled to update BDx run modules.

IMPORTANT! Uninstalling the BDx Utility removes all existing BDx objects from the Data Collection Software database.

1. Power on the instrument and the computer with your Data Collection Software.
2. Exit the Data Collection Software if it is running.
3. Select **Start** ▶ **Programs** ▶ **Applied Biosystems™** ▶ **BDx Utility** ▶ **Uninstall BDx**.
4. Follow the prompts in the **Uninstaller** wizard.
All existing BDx run modules are removed.
5. Double-click the **bdx1.0.1updater.exe** file to run the BDx Utility.
6. Follow the prompts in the **Support Files Installer** wizard.
The BDx Utility installs new BDx run modules and calibrates the run modules based on the instrument's autosampler calibration.

Update BDx run modules

Run the BDx Utility to update BDx run modules after Data Collection Software installation and every time the instrument autosampler is recalibrated.

1. Exit the Data Collection Software if it is running. Do not shut down the data-collection computer.
2. Power on the instrument.
3. Select **Start** ▶ **Programs** ▶ **Applied Biosystems™** ▶ **BDx Utility** ▶ **Update BDx Utility**.



Uninstall BDx run modules

IMPORTANT! Uninstalling a BDx run module also removes any instrument protocols and plate records that use the run module. Run folders, containing AB1 files that have been extracted to the hard drive, are not removed.

1. Power on the instrument and the computer with the Data Collection Software.
2. Exit the Data Collection Software if it is running.
3. Select **Start** ▶ **Programs** ▶ **Applied Biosystems™** ▶ **BDx Utility** ▶ **Uninstall BDx**.
4. Follow the prompts in the **Uninstaller** wizard.



Robotic Pipetting method

This section provides general information to integrate the BigDye XTerminator™ Purification Kit procedure into a robotic pipetting workflow. For detailed automation instructions, see the appropriate instrument user guide.

Compatible instruments

The BigDye XTerminator™ Purification Kit is compatible with the following automated workstations:

- Biomek™ i7 Automated Workstation
- Biomek™ FX^P Automated Workstation
- Biomek™ FX Automated Workstation
- Biomek™ NX Automated Workstation

Purification procedure

IMPORTANT! Keep the Premix Solution thoroughly mixed throughout the pipetting procedure.

See the Biomek™ Liquid Handler User's Manual to set up and run the appropriate method on the instrument.

1. Set up the method for the instrument.
2. Prepare the Premix Solution in an appropriately-sized bottle as described—See “Prepare the Premix Solution” on page 14.
3. Mix the Premix Solution by inversion 10 times, or until the solution is homogeneous, then transfer to a reservoir.
4. Run the method to aspirate, then dispense the Premix Solution.
Refill the solution reservoir as needed.
5. When the run is complete, seal the plate according to your sealing method.
 - Using a MicroAmp™ Clear Adhesive Film—Proceed to “Seal the reaction plate” on page 16.
 - Using a heat seal—Apply a heat seal at 160°C for 1.5 seconds, then proceed to “Vortex, then centrifuge the reaction plate” on page 16.



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.
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Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).

- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
 - Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
 - Manipuler les déchets chimiques dans une sorbonne.
 - Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
 - Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
 - Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
 - Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
 - **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.
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Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>
 - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
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Documentation and support

Related documentation

| Document | Publication number |
|---|--|
| <i>BigDye XTerminator™ Purification Kit Quick Reference</i> | 4383427 |
| <i>DNA Sequencing by Capillary Electrophoresis Chemistry Guide Second Edition</i> | 4305080 |
| <i>Troubleshooting Sanger sequencing data</i> | MAN0014435 |
| <i>SeqStudio™ Genetic Analyzer Instrument and Software User Guide</i> ^[1] | MAN0018646 |
| <i>SeqStudio™ Genetic Analyzer Instrument and Software User Guide</i> ^[2] | MAN0016138 |
| <i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 User Guide</i> | 100031809 |
| <i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide</i> | 100079380 |
| <i>User Guide: Applied Biosystems™ 3730/3730xl DNA Analyzer</i> | 4331468 |
| <i>Applied Biosystems™ 3730/3730xl DNA Analyzer Chemistry Guide</i> | 4331467 |
| <i>3130/3130xl Genetic Analyzers Getting Started Guide</i> | 4477796 |
| <i>310 Genetic Analyzer Manual for Windows™</i> | 4317588 |
| Beckman Coulter™ documentation: ^[3] <ul style="list-style-type: none"> • <i>Biomek™ Liquid Handler User's Manual</i> • <i>Biomek™ Software User's Manual</i> | <ul style="list-style-type: none"> • Beckman Coulter 987834 • Beckman Coulter 987835 |
| <i>BigDye™ Terminator v1.1 Cycle Sequencing Kit User Guide</i> | 4337036 |
| <i>BigDye™ Terminator v3.1 Cycle Sequencing Kit User Guide</i> | 4337035 |

^[1] For use with SeqStudio™ Data Collection Software v1.2.

^[2] For use with SeqStudio™ Data Collection Software v1.1.4.

^[3] Provided with the Biomek™ software (Start ▶ All Programs ▶ Beckman Coulter™ ▶ Manuals).



Customer and technical support

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- Worldwide contact telephone numbers
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- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

