Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific MagJET NGS Cleanup and Size Selection Kit

#K2821, #K2822, #K2823, #K2828

Read Storage information (p. 4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ NGS Cleanup and Size Selection Kit is qualified by isolation of a 300 bp DNA fragment using the size-selection procedure outlined in the manual. The yield and quality of the isolated DNA fragment is determined by agarose gel electrophoresis.

Quality authorized by:  **Jurgita Žilinskienė**

Rev. 1

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COMPONENTS OF THE KIT

MagJET NGS Cleanup and Size Selection Kit	#K2828 10 preps*	#K2821 96 preps*	#K2822 4 × 96 preps*	#K2823 10 × 96 preps*
MagJET Magnetic Beads	110 µL	0.6 mL	2 × 1.2 mL	6 mL
Binding Buffer (concentrated)	8 mL	55 mL	210 mL	3 × 175 mL
Wash Buffer (concentrated)	3 mL	25 mL	2 × 45 mL	4 × 60 mL
Elution Buffer	2 × 1.5 mL	18 mL	2 × 45 mL	3 × 60 mL
DNA Fragment Mix	1 mL	1 mL	2 × 1 mL	5 × 1 mL

*The prep size refers to one cleanup application. See Table 1 for size compatibility with different adapter removal and size selection applications.

Table 1. Kit size compatibility with different applications.

Product	Cat. No.	MagJET Magnetic Beads	Cleanup application	Adapter removal application	Size selection application
MagJET NGS Cleanup and Size Selection Kit	**#K2828**	110 µL	10 preps	5 preps	3 preps
	#K2821	0.6 mL	96 preps	48 preps	32 preps
	#K2822	2 × 1.2 mL	384 preps	192 preps	128 preps
	#K2823	6 mL	960 preps	480 preps	320 preps

STORAGE

Store MagJET Magnetic Beads at 4 °C and DNA Fragment Mix at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

DESCRIPTION

The MagJET NGS Cleanup and Size Selection Kit is designed for highly efficient and robust DNA fragment library cleanup from a variety of enzymatic reaction mixtures including PCR, ligation, adapter addition and DNA end-repair reaction mixes. The kit utilizes high-capacity MagJET paramagnetic bead technology that ensures efficient recovery of DNA fragment libraries within a desired fragment length range. The purified DNA fragments are free of any next-generation sequencing workflow inhibiting components, such as sequencing adapters, primer dimers, unincorporated nucleotides, enzymes or salts, and are ready-to-use in downstream NGS applications. No prior DNA fragment library purification is required before cleanup and size-selection procedures. The kit is suitable for cleanup or size selection of 5 ng to 5 µg of DNA fragment library. The Cleanup and Adapter removal protocols can easily be automated for higher throughput on automatic platforms such as Thermo Scientific™ KingFisher™ Flex and Thermo Scientific™ KingFisher™ Duo.

PRINCIPLE

The MagJET NGS Cleanup and Size Selection Kit utilizes a highly efficient MagJET magnetic particle-based technology for nucleic acid isolation. The entire DNA fragment cleanup and size selection process combines the simple steps of binding DNA to the magnetic beads, washing and elution. During the initial cleanup steps a DNA fragment library, in the presence of Binding Buffer and isopropanol, is captured on MagJET Magnetic Beads, whereas all other unwanted reaction components remain in the buffer and are discarded. A subsequent washing step removes remaining reaction contaminants. Finally, the purified DNA fragment library is eluted in the Elution Buffer. When using size-selection protocols, additional binding and elution steps are used to isolate the DNA library of the desired fragment length.

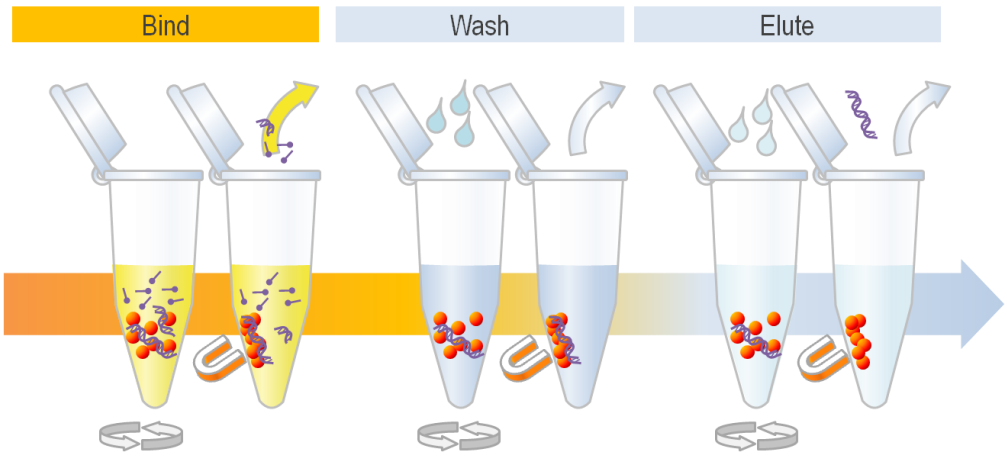

Figure 1. Principal scheme of MagJET nucleic acid purification technology

Table 2. Kit specifications

	Protocol	Sample type	Specifications
A	**Cleanup protocol**	PCR, end-repair, DNA tailing reaction mixtures, cleanup from fragmentation buffers.	>97% removal of DNA fragments shorter than 150 bp; > 70% recovery of fragments longer than150 bp .
B	**Adapter removal protocol**	Cleanup from sequencing adapter addition (ligation) reaction mixes.	>99% removal of fragments shorter than 150 bp; > 70% recovery of fragments longer than 150 bp .
C	**Size-selection protocol**	DNA libraries in various enzymatic reaction mixtures and buffers.	

IMPORTANT NOTES

To avoid precipitates, prepare a fresh Binding Mix before each use! Add the exact amount of 100% isopropanol to the final concentration of 36% (v/v) (see Table 3 for the guidelines). First add isopropanol, then add Binding Buffer. Mix well by inverting tube several times, shaking or vortexing**.** Prepared Binding Mix must be used within 24 hours.

⚠ **Important: To obtain reproducible size selection results a precise volume of isopropanol and Binding Buffer must be used for Binding Mix preparation!** Make sure well-calibrated pipettes and new pipette tips are used each time when isopropanol and Binding Buffer are used to prepare the Binding Mix. We strongly recommend using well-calibrated laboratory scales for the preparation of Binding Mix for 10 or more samples (see Table 3).

Table 3. Binding Mix preparation

Number of samples		Total Binding Mix volume*	100 % isopropanol volume (mass)	Binding Buffer volume (mass)	Laboratory tool for Binding Mix preparation
for Cleanup protocol	for Adapter removal protocol				
2	1	1,600 µL	576 µL	1,024 µL	Calibrated pipette
4	2	3,200 µL	1,152 µL	2,048 µL	
6	3	4,800 µL	1,728 µL	3, 072 µL	
8	4	6,400 µL	2,304 µL	4,096 µL	
10	5	8,000 µL	2,880 µL	5,120 µL	
20	10	16,000 µL	4.53 g	11.02 g	Calibrated laboratory scales (0,01±0,02 g)
192	96	155,000 µL	43.86 g	106.74 g	

- Add the indicated volume of ethanol (96-100%) to Wash Buffer (concentrated) prior to first use:

MagJET NGS Cleanup and Size Selection Kit	10 preps #K2828	1 × 96 preps #K2821	4 × 96 preps #K2822	10 × 96 preps #K2823
Wash Buffer (conc.)	3 mL	25 mL	2 × 45 mL	4 × 60 mL
Ethanol (96-100%)	9 mL	75 mL	2 × 135 mL	4 × 180 mL
Total volume	**12 mL**	**100 mL**	**2 × 180 mL**	**4 × 240 mL**

- **Thoroughly mix the MagJET Magnetic Beads to fully resuspend the particles in the storage solution before each use.**
- The protocols are validated for DNA libraries of fragment lengths up to 1,000 bp.
- All steps should be carried out at room temperature.
- Always use freshly prepared Binding Mix.
- Always premix MagJET Magnetic Beads and Binding Mix before adding the sample.
- To ensure the best DNA yields **do not** lose any magnetic beads during the procedures and **do not** shorten incubation times described.
- Check all solutions in the kit for any salt precipitates before each use. Re-dissolve precipitates by warming the solution at 37 °C, and then equilibrate to room temperature (20 ± 5 °C).
- Instructions are provided to purify the DNA samples in 100 µL volume. Supplement sample volume with **water or TE buffer to 100 µL** if necessary. If you wish to process < 100 µL be sure to scale-down the volume of Binding Mix but keep the volume of MagJET Magnetic Beads the constant (5 µL).
- When using protocols for higher throughput, pre-mix Binding Mix and MagJET Magnetic Beads before use. Prepare sufficient amount of the mixture plus 10% extra.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Calibrated pipettes and pipette tips
- Vortex
- Microcentrifuge
- LoBind™ Tubes 1.5 mL (Cat. #022431021, Eppendorf)
- Disposable gloves
- Automatic magnetic particle processor and consumables *or*
- Magnetic rack (for optimal results use Thermo Scientific™ MagJET™ Separation Rack, Cat. #MR01/2)
- Ethanol 96-100%, molecular biology grade
- Isopropanol 100%, molecular biology grade
- Calibrated laboratory scales (accuracy: 0.01±0.02 g)
- Agarose gel (2%)

PROTOCOL SELECTION GUIDE

The MagJET NGS Cleanup and Size Selection Kit provides two specialized protocols optimized for fast DNA fragment library cleanup and NGS adapter removal. An additional guide is provided for size selection applications.

- The Cleanup protocol is recommended for fast purification of DNA fragment libraries from PCR and DNA end-conversion reaction mixtures and buffers.
- The Adapter removal protocol is highly efficient at removing large quantities of sequencing adapters from the adapter addition reaction mixtures.
- The Size selection guide provides recommendations for selection of required DNA fragment library size range.

Protocol selection guide:

Sample type	Sample quantity	Throughput per run	KingFisher Flex Instrument	KingFisher Duo Instrument	Manual processing	MagJET purification protocol	Page
Cleanup PCR, DNA end-repair, DNA tailing reaction mixtures and buffers, sonication buffers.	5 ng - 5 µg	Variable	-	-	●	**Protocol A**	page 9
		Up to 12	-	●	-	**Protocol E**	page 20
		Up to 96	●	-	-	**Protocol D**	page 18
Adapter removal Adapter addition and ligation mixes.	5 ng - 5 µg	Variable	-	-	●	**Protocol B**	page 10
		Up to 12	-	●	-	**Protocol G**	page 24
		Up to 96	●	-	-	**Protocol F**	page 22
Size-selection DNA library samples in various enzymatic reaction mixtures and buffers.	50 ng - 5 µg	Variable	-	-	●	**Protocol C**	page 12

CLEANUP AND SIZE SELECTION INSTRUCTIONS

A. Cleanup Protocol

This protocol is suitable for DNA fragment library cleanup from DNA fragmentation buffers, DNA blunting, tailing or PCR reaction mixes. The protocol is validated for physically fragmented DNA libraries of fragment lengths up to 1,000 bp.

Note:

- **Prepare fresh Binding Mix as described in page 6.**
- **Before each use, mix MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution.**

1	Mix 5 µL of **MagJET Magnetic Beads** with 700 µL of **Binding Mix**.
2	Transfer exactly 100 µL DNA sample to a microcentrifuge tube with pre-mixed MagJET Magnetic Beads and Binding Mix.
3	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, pulse-spin the tube to collect all the drops and incubate at room temperature for 5 minutes.
4	Briefly spin down the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and discard the supernatant by using a pipette. Make sure that all supernatant is removed. **Note**: If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
5	Add 400 µL of **Wash Solution** (supplemented with ethanol). Mix by vortexing, spin the tube briefly, and place it back in the magnetic rack for 1 - 2 minutes. When solution clears carefully remove and discard the supernatant by using a pipette.
6	Repeat *step 5.*
7	To remove residual Wash Solution, pulse-spin the tube, place it back in the magnetic rack for 1 minute, and carefully remove any remaining supernatant with a pipette without disturbing the pellet.
8	Keeping the tube on the magnet, air dry the magnetic particles at room temperature for 5 minutes or until there are no droplets of Wash Solution left on the walls of the tube.
9	Remove the tube from the magnet and add 10-50 µL of **Elution Buffer.**
10	Mix by vortexing, spin down the tube to collect all the drops and incubate at room temperature for 1 minute.
11	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and transfer the eluate to a storage tube. **Note:** If the pellet of magnetic particles was disturbed or eluate is not clear enough, mix the sample by gentle pipetting and let the beads settle to the magnet again.

B. Adapter Removal Protocol

This protocol is suitable for DNA fragment library cleanup after a sequencing adapter addition reaction. The amount of sequencing adapters in the ligation reaction **should not exceed 7 µg**. The protocol is validated for physically fragmented DNA libraries of fragment lengths up to 1,000 bp.

Note:

- **Prepare fresh Binding Mix as described in page 6.**
- **Before each use mix MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution.**

1	Mix 5 µL of **MagJET Magnetic Beads** with 700 µL of **Binding Mix**.
2	Transfer exactly 100 µL DNA sample to a microcentrifuge tube with pre-mixed MagJET Magnetic Beads and Binding Mix.
3	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, pulse-spin the tube to collect all the drops and incubate at room temperature for 5 minutes.
4	Briefly spin down the tube to collect droplets. Place the tube in the magnetic rack for 2 - 3 minutes or until the beads have formed a tight pellet. Keeping the tube on the magnet, carefully remove and discard the supernatant by using a pipette. Make sure that all supernatant is removed. **Note:** To remove residual solution pulse-spin the tube and place it in the magnetic rack again until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and discard the supernatant by using a pipette. If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
5	With the reaction tube still on the magnet, add 105 µL of Elution Buffer. Mix by vortexing, spin briefly to collect droplets and incubate at room temperature for 1 minute.
6	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet.
7	Mix 5 µL of **fresh** MagJET Magnetic Beads with 700 µL **Binding Mix** in a new tube.

8	Without removing the microcentrifuge tube from the magnetic rack, transfer exactly 100 µL of **supernatant** from *step 6* to the new tube from *step 7*, pre-filled with a mixture of **MagJET Magnetic Beads** and **Binding Mix.** Discard used magnetic beads. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
9	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, spin down the tube to collect all the drops and incubate at room temperature for 5 minutes.
10	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and discard the supernatant by using a pipette. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
11	Add 400 µL of Wash Solution (supplemented with ethanol). Mix by vortexing, spin the tube briefly and place it back in the magnetic rack for 1-2 minutes. When solution clears carefully remove and discard the supernatant by using a pipette.
12	Repeat *step 11.*
13	To remove residual Wash Solution, pulse-spin the tube, place it back in the magnetic rack for 1 minute, and carefully remove any remaining supernatant with a pipette without disturbing the pellet.
14	Keeping the tube on the magnet, air dry the magnetic particles at room temperature for 5 minutes or until there are no droplets of Wash Solution left on the walls of the tube.
15	Remove the reaction tube from the magnet and add 10-50 µL of Elution Buffer.
16	Mix by vortexing, spin down the tube to collect all the drops and incubate at room temperature for 1 minute.
17	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and transfer the eluate to a storage vessel. **Note:** If the pellet of magnetic particles was disturbed or eluate is not clear enough, mix the sample by gentle pipetting and let the beads settle to the magnet again.

C. Size Selection Guide

The following guide provides recommendations for size selection of a DNA library of a specific fragment length range. The provided workflow is compatible with unpurified DNA library samples in various enzymatic reaction mixtures and buffers, therefore no prior cleanup or adapter removal step is required before the size selection procedure.

The principle of the procedure is based on the binding of DNA fragments to the magnetic beads in the presence of isopropanol and Binding Buffer. During the initial cleanup steps a DNA fragment library is captured on the magnetic beads, whereas DNA fragments shorter than 150 bp remain in the buffer and are discarded. In later steps DNA fragments that are longer than desired are captured on magnetic beads while the remaining fragments are transferred to a new tube with new magnetic beads. During third binding step, fragments that are shorter than desired are not bound to the beads. After subsequent wash and elution steps a pure DNA library of required fragment length is obtained (Figure 2).

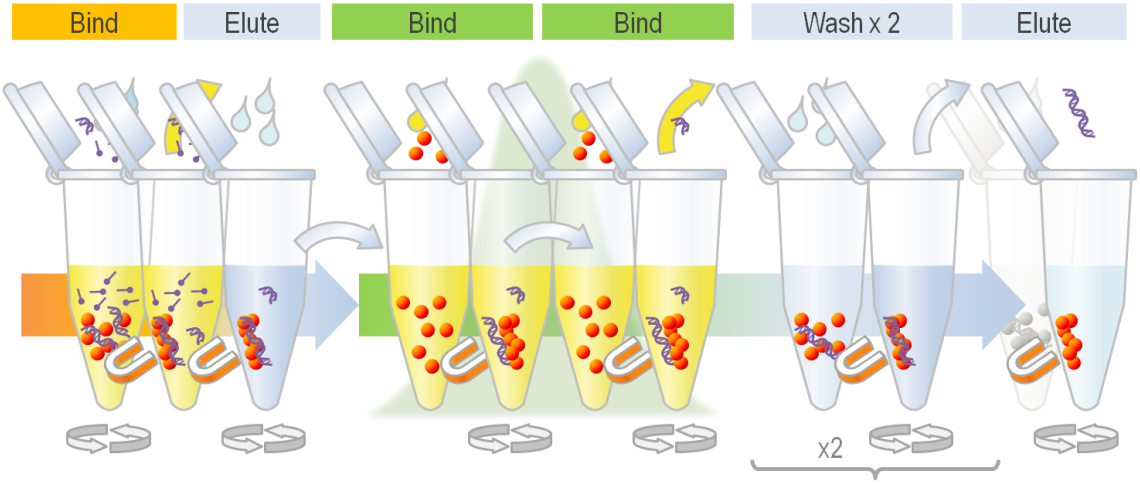

Figure 2. MagJET size selection procedure scheme

As a general rule, increasing volume of Binding Mix (Table 4) will increase the efficiency of capturing larger DNA molecules on magnetic beads, resulting in the recovery of shorter fragments after purification procedure due to transfer of unbound fraction to new beads. Figure 3 illustrates this relationship.

Important: To obtain reproducible size selection results a precise volume of isopropanol and Binding Buffer must be used for Binding Mix preparation. Make sure well-calibrated pipettes are used during all steps of the size selection procedure.

Before continuing to the size selection procedure, we strongly recommend testing the prepared Binding Mix by performing a quick Calibration protocol. The Calibration protocol allows for fine-tuning the required volume of Binding Mix for exact size selection of required DNA fragment range. For the subsequent size selection procedure use the determined Binding Mix volume that produces the highest yield of target fragment in the Calibration protocol. Additional calibration is unnecessary if the same Binding Mix conditions and equipment are used during next size selection procedure. Prepared Binding Mix must be used within 24 hours.

Calibration protocol

The Calibration protocol is a quick procedure intended for identification of the optimal Binding Mix volume for the subsequent size selection procedure.

Note:

- Use the same Binding Mix for both the Calibration protocol and the Size Selection procedure.
- Use the same pipettes and pipetting techniques for both the Calibration protocol and the Size selection procedure.
- Mix MagJET Magnetic Beads well to fully resuspend particles in the storage solution before each use.
- Make sure to always fully discard the Binding Mix from the pipette tip during the procedure. For best results we recommend using Thermo Scientific™ F1-ClipTip™ pipettes.
- We recommend using a fragmented DNA sample and Agilent 2100 Bioanalyzer for the calibration. If spare fragmented DNA is not available, use the included DNA Fragment Mix and analyze results using agarose gel electrophoresis. DNA must be dissolved in molecular biology grade water, 10 mM Tris (pH 7.5-8) or TE buffer for Calibration protocol, DNA amount should not exceed 5 µg. Always use exact (100 µL) sample volume.

1	Mix 5 µL of **MagJET Magnetic Beads** with each of the different volumes of **Binding Mix** indicated in **Table 4, Binding Volume X**. **Note:** To obtain reproducible size selection results new pipette tips should be used for each sample when Binding Mix is added.
2	Transfer exactly 100 µL **DNA Fragment Mix or fragmented DNA** to a microcentrifuge tube with pre-mixed MagJET Magnetic Beads and Binding Mix.
3	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, pulse-spin the tube to collect all the drops and incubate at room temperature for 2 minutes.
4	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Target DNA is in solution. **Note:** Target DNA is in solution.
5	In a new tube mix 5 µL of **fresh** MagJET Magnetic Beads with 100 µL of **Binding Mix.** **Note:** To obtain reproducible size selection results new pipette tips should be used for each sample when Binding Mix is added.

6	Without removing the microcentrifuge tube from the magnetic rack, transfer the **supernatant** from *step 4* to the new tube from *step 5*, pre-filled with a mixture of **MagJET™ Magnetic Beads and Binding Mix.** Discard used magnetic beads. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
7	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, spin down the tube to collect all the drops and incubate at room temperature for 2 minutes.
8	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and discard the supernatant by using a pipette. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again. Target DNA is on beads.
9	To remove residual solution, pulse-spin the tube, place it back in the magnetic rack for 1 minute, and carefully remove any remaining supernatant with a pipette without disturbing the pellet.
10	Remove the reaction vessel from the magnet and add 15 µL of Elution Buffer, mix by vortexing.
11	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and transfer the eluate to a storage vessel. **Note:** If the pellet of magnetic particles was disturbed or eluate is not clear enough, mix the sample by gentle pipetting and let the beads settle to the magnet again.
12	Use 5-10 µL of eluate to analyze results using agarose (2%) gel electrophoresis (TAE or TBE) *or* use the Agilent 2100 Bioanalyzer. For DNA library size selection choose the Binding Mix volume that produces the highest yield of target fragment.

Table 4. Recommended Binding Mix volumes for Calibration protocol *Step 1*.

Binding Volume X	
Average desired DNA fragment length, bp	**Binding Mix, µL**
200 bp	400, 450, 500, 550, 600 µL
300 bp	300, 350, 400, 450, 500 µL
400 bp	250, 300, 350, 400, 450 µL
500 bp	200, 250, 300, 350, 400 µL
700 bp	150, 200, 250, 300, 350 µL

Interpretation of Calibration protocol results obtained using DNA Fragment Mix

In the example below the DNA Fragment Mix was first used in the Calibration protocol. Various Binding Mix volumes were titrated to identify the optimal Binding Mix volume required for size selection of a target DNA fragment range, e. g., a 250 µL Binding Mix volume resulted in highest yield of 700 bp DNA fragment (Figure 3). The identified Binding Mix volumes were used in the Size selection procedure with fragmented DNA and resulted average peak sizes correlating to the greatest yield found from the Calibration protocol for each Binding Mix volume (Figure 4).

For fine-tuning the required DNA fragment size selection peak you may titrate Binding Mix used in first protocol step every 25 µL, especially when DNA target fragment is longer than 400 bp, e. g., if target fragment length is 500 bp you can use 250-350 µL range of Binding Mix (after first testing).

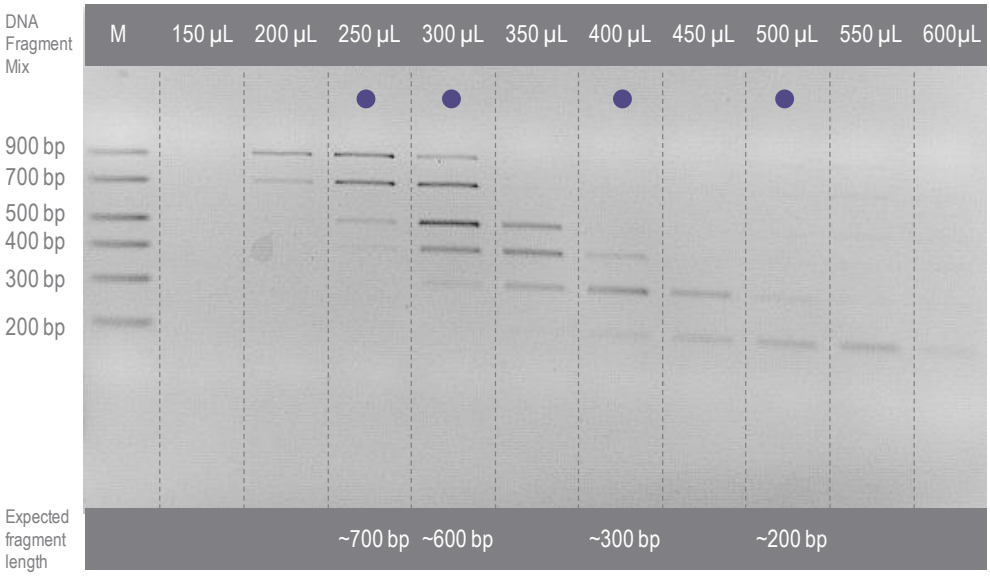

Figure 3. Example of Calibration protocol results using 150-600 µL Binding Mix volumes.

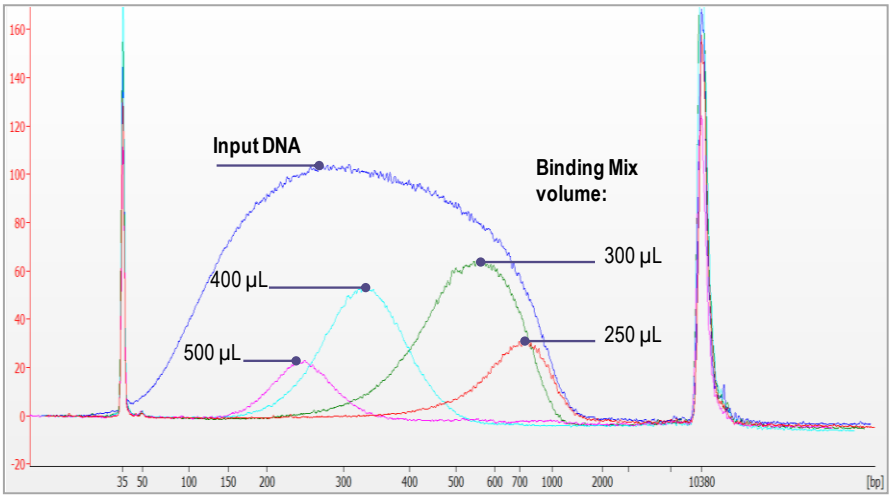

Figure 4. Size Selection procedure performed with identified Binding Mix volumes.

Size Selection Procedure

1	Mix 5 µL of **MagJET Magnetic Beads** with 700 µL of **Binding Mix**.
2	Transfer exactly 100 µL DNA sample to a microcentrifuge tube with pre-mixed MagJET Magnetic Beads and Binding Mix.
3	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, pulse-spin the tube to collect all the drops and incubate at room temperature for 5 minutes.
4	Spin briefly to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and **discard the supernatant** by using a pipette. Make sure that all supernatant is removed. **Note:** To remove residual solution pulse-spin the tube and place it in the magnetic rack until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and discard the supernatant by using a pipette. If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again. Target DNA stays on beads, steps 1-4 remove excess of adapters.
5	With the reaction tube still on the magnet, add 105 µL of Elution Buffer. Mix by vortexing, spin briefly to collect droplets and incubate at room temperature for 1 minute.
6	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Target DNA is in solution.
7	In a new tube mix 5 µL of **fresh** MagJET Magnetic Beads with an appropriate **Binding Mix** volume determined by the Calibration protocol (*see* **Table 4, Binding Volume X**). **Note:** To obtain reproducible size selection results new pipette tips should be used for each sample when Binding Mix is added.
8	Without removing the microcentrifuge tube from the magnetic rack, transfer exactly 100 µL of **supernatant** from *step 6* to the new tube from *step 7*, pre-filled with a mixture of **MagJET Magnetic Beads and Binding Mix. Discard used magnetic beads**. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
9	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, spin down the tube to collect all the drops and incubate at room temperature for 5 minutes.

10	Briefly spin down the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Note: Target DNA is in solution. Steps 5-10 remove DNA larger than target DNA.
11	In a new tube mix 5 µL of **fresh** MagJET Magnetic Beads with 100 µL of **Binding Mix**. **Note:** To obtain reproducible size selection results new pipette tips should be used for each sample when Binding Mix is added.
12	Without removing the microcentrifuge tube from the magnetic rack, transfer the **supernatant** from *step 10* to the new tube from *step 11*, pre-filled with a mixture of **MagJET Magnetic Beads and Binding Mix.** Discard used magnetic beads. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again.
13	Mix the total reaction volume by vortexing until a homogenous suspension is obtained, spin down the tube to collect all the drops and incubate at room temperature for 5 minutes.
14	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Keeping the reaction vessel on the magnet, carefully remove and discard the supernatant by using a pipette. **Note:** If the pellet of magnetic particles was disturbed, mix the sample and let the beads settle to the magnet again. Target DNA is on beads.
15	Add 400 µL of Wash Solution (supplemented with ethanol). Mix by vortexing, spin the tube briefly, and place it back in the magnetic rack for 1-2 minutes. When solution clears carefully remove and discard the supernatant by using a pipette.
16	Repeat *step 15*.
17	To remove residual Wash Solution, pulse-spin the tube, place it back in the magnetic rack for 1 minute, and carefully remove any remaining supernatant with a pipette without disturbing the pellet.
18	Keeping the tube on the magnet, air dry the magnetic particles at room temperature for 5 minutes or until there will be no droplets of Wash Solution left on the walls of the tube.
19	Remove the reaction vessel from the magnet and add 10-50 µL of Elution Buffer.
20	Mix by vortexing, spin briefly to collect all the drops and incubate at room temperature for 1 minute.
21	Pulse-spin the tube to collect droplets. Place the tube in the magnetic rack for 2-3 minutes or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and transfer the eluate to a storage vessel. **Note:** If the pellet of magnetic particles was disturbed or eluate is not clear enough, mix the sample by gentle pipetting and let the beads settle to the magnet again.

D. Cleanup Protocol for KingFisher Flex Magnetic Particle Processor

The following protocol provides instructions for DNA purification using Thermo Scientific™ KingFisher™ Flex with 96-pin magnet head and Thermo Scientific™ Microtiter™ deep well 96 and Thermo Scientific™ KingFisher™ Flex 96 plates. This protocol is suitable for DNA fragment library cleanup from DNA fragmentation buffers, DNA blunting, tailing or PCR reaction mixes. The protocol is validated for physically fragmented DNA libraries of fragment lengths up to 1,000 bp.

Notes:

- When using the MagJET NGS Cleanup and Size Selection Kit for the first time, prepare the working solution of Wash Solution as described on page 6.
- Prepare a fresh Binding Mix as described in page 6.
- Before each use shake MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution.
- Mix Binding Mix and MagJET Magnetic Beads before filling in the plate. In such case use 705 µL of this mixture per well.
- Transfer the NGS_Cleanup_Flex protocol file to the KingFisher Flex instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Cleanup and Size Selection Kit can be found on the product web page on www.thermoscientific.com/onebio
- Ensure that you are using the KingFisher Flex 96-pin magnet head.

1	Obtain three empty Microtiter deep well 96 plates, two Thermo Scientific KingFisher Flex 96 KF plates and one Thermo Scientific KingFisher Flex 96-Tip Comb.
2	Add 705 µL of **Binding Mix and MagJET Magnetic Beads mixture** to **Sample plate.** **Note.** Resuspend magnetic bead suspension well before first use**.**
3	Add the following reagents to **plates 2, 3 and 4** as described in Table 5.
4	Transfer 100 µL DNA sample to the **Sample plate** with pre-mixed MagJET Magnetic Beads and Binding Mix.
5	Switch on the KingFisher Flex instrument and start the **NGS_Cleanup_Flex** protocol. Insert the plates into the instrument as indicated on the KingFisher Flex display. Make sure the plates are placed in the correct orientation.
6	After the run is finished, remove the plates and turn off the instrument. Transfer the eluate (containing DNA) to a new, clean vessel. The purified DNA is ready for use in downstream applications or can be stored at -20 °C for later use.

Table 5. Sample and reagent setup scheme.

Plate name and type	Plate number	Plate name	Content	Sample/reagent volume per well
Microtiter deep well 96 plate	1	Sample	MagJET Magnetic Beads	5 µL
			Binding Mix	700 µL
			DNA sample	100 µL
	2	Wash_1	Wash solution	400 µL
	3	Wash_2	Wash solution	400 µL
KingFisher Flex 96 KF plate	4	Elution	Elution Buffer	50 µL
	5	Tip	96-tip comb	*Empty*

E. Cleanup Protocol for KingFisher Duo Magnetic Particle Processor

The following protocol provides instructions for DNA purification using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate. This protocol is suitable for DNA fragment library cleanup from DNA fragmentation buffers, DNA blunting, tailing or PCR reaction mixes. The protocol is validated for physically fragmented DNA libraries of fragment lengths up to 1,000 bp.

Notes:

- When using the MagJET NGS Cleanup and Size Selection Kit for the first time, prepare the working solution of Wash Solution as described on page 6.
- Prepare fresh Binding Mix as described in page 6.
- Before each use shake MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution.
- Mix Binding Mix and MagJET Magnetic Beads before filling in the plate. In such case use 705 µL of this mixture per well.
- Transfer the NGS_Cleanup_Duo protocol file to the KingFisher Duo instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Cleanup and Size Selection Kit can be found on the product web page on www.thermoscientific.com/onebio
- Ensure that you are using the KingFisher Duo 12-pin magnet head and heating block.

1	Obtain one empty Thermo Scientific Microtiter deep well 96 plate, one Thermo Scientific KingFisher Duo elution strip and one Thermo Scientific KingFisher Duo 12-Tip strip.
2	Add 705 µL of **Binding Mix and MagJET Magnetic Beads mixture** to **row A** **Note.** Resuspend magnetic bead suspension well before first use**.**
3	Add the following reagents to **rows C, D** and **Elution Strip** as described in Table 6. Note that **row B** is reserved for the tip and should be left empty. Note that rows **E-H** are left empty. Add Thermo Scientific KingFisher Duo 12-Tip strip to row B.
4	Transfer 100 µL DNA sample to row A with pre-mixed MagJET Magnetic Beads and Binding Mix.
5	Switch on the KingFisher Duo instrument and start the **NGS_Cleanup_Duo** protocol. Insert the **NGS Cleanup** plate and **Elution Strip** into the instrument as indicated on the KingFisher Duo display. Make sure that the **Elution Strip** is placed in the correct orientation into the elution block. Ensure that the perforated end is facing towards the user.
6	After the run is finished, remove the plate and turn off the instrument. Transfer the eluate (containing DNA) to a new clean vessel. The purified DNA is ready for use in downstream applications or can be stored at -20 °C for later use.

Table 6. Sample and reagent setup scheme.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
NGS Cleanup Plate (Microtiter deep well 96 plate)	A	Sample	MagJET Magnetic Beads	5 µL
			Binding Mix	700 µL
			DNA sample	100 µL
	B	Tip	12-tip comb	*Empty*
	C	Wash_1	Wash solution	400 µL
	D	Wash_2	Wash solution	400 µL
	E	*Empty*	*Empty*	*Empty*
	F	*Empty*	*Empty*	*Empty*
	G	*Empty*	*Empty*	*Empty*
	H	*Empty*	*Empty*	*Empty*
KingFisher Duo Elution strip	-	Elution	Elution Buffer	50 µL

F. Adapter Removal Protocol for KingFisher Flex Magnetic Particle Processor

The following protocol provides instructions for DNA purification using KingFisher Flex with 96-pin magnet head and Microtiter deep well 96 and KingFisher Flex 96 plates. This protocol is suitable for DNA fragment library cleanup after the sequencing adapter addition reaction. The amount of sequencing adapters in the ligation reaction **should not exceed 7 µg**. The protocol is validated for physically fragmented DNA libraries of fragment lengths up to 1,000 bp.

Notes:

- When using the MagJET NGS Cleanup and Size Selection Kit for the first time, prepare the working solution of Wash Solution as described on page 6.
- Prepare fresh Binding Mix as described in page 6.
- Before each use shake MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution.
- Mix Binding Mix and MagJET Magnetic Beads before filling in the plate. In such case use 705 µL of this mixture per well.
- Transfer the NGS_Adapter_Flex protocol file to the KingFisher Flex instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Cleanup and Size Selection Kit can be found on product web page on www.thermoscientific.com/onebio
- Ensure that you are using the KingFisher Flex 96-pin magnet head.

1	Obtain four empty Thermo Scientific Microtiter deep well 96 plates, two Thermo Scientific KingFisher Flex 96 KF plates and one Thermo Scientific KingFisher Flex 96-Tip Comb.
2	Add 705 µL of **Binding Mix and MagJET Magnetic Beads mixture** to **Sample plate (1)**. **Note.** Resuspend magnetic bead suspension well before first use**.**
3	Add 100 µL of Elution Buffer to **Elution_1_and_Rebind plate (2)** as indicated in Table 7, 2.1.
4	Add the following reagents to **plates 3-5** as described in Table 7.
5	Transfer 100 µL DNA sample to the Sample plate (1) with pre-mixed MagJET Magnetic Beads and Binding Mix.
6	Switch on the **KingFisher Flex** instrument and start the **NGS_Adapter_Flex** protocol. Insert the plates into the instrument as indicated on the KingFisher Flex display. Make sure the plates are placed in the correct orientation.
7	When the KingFisher Flex pauses at the **rebind** step (approximately 12 minutes after starting the run), remove the **Elution_1_and_Rebind** plate (2) from the instrument and add 705 µL of **Binding Mix and MagJET Magnetic Beads mixture** per well to rebind the DNA (Table 7, 2.2).

8	Place the **Elution_1_and_Rebind** plate back into the instrument and press OK. After the pause, the protocol will continue through to completion.
9	After the run is finished, remove the plates and turn off the instrument. Transfer the eluate from **Elution_2 plate** (containing DNA) to a new, clean vessel. The purified DNA is ready for use in downstream applications or can be stored at -20°C for later use.

Table 7. Sample and reagent setup scheme.

Plate name and type	Plate number	Plate name	Content	Sample/reagent volume per well
Microtiter deep well 96 plate	1	Sample	MagJET Magnetic Beads	5 µL
			Binding Mix	700 µL
			DNA sample	100 µL
	2.1	Elution_1_and_Rebind	Elution Buffer	100 µL
	2.2	Elution_1_and_Rebind	MagJET Magnetic Beads	5 µL
			Binding Mix	700 µL
	3	Wash_1	Wash solution	400 µL
	4	Wash_2	Wash solution	400 µL
KingFisher Flex 96 KF plate	5	Elution_2	Elution Buffer	50 µL
	6	Tip	96-tip comb	*Empty*

G. Adapter Removal Protocol for KingFisher Duo Magnetic Particle Processor

The following protocol provides instructions for DNA purification using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate. This protocol is suitable for DNA fragment library cleanup after the sequencing adapter addition reaction. The amount of sequencing adapters in the ligation reaction **should not exceed 7 µg**. The protocol is validated for physically fragmented DNA libraries of fragment lengths up to 1,000 bp.

Notes:

- When using the MagJET NGS Cleanup and Size Selection Kit for the first time, prepare working solution of Wash Solution as described on page 6.
- Prepare fresh Binding Mix as described in page 6.
- Before each use shake MagJET Magnetic Beads thoroughly to fully resuspend the particles in the storage solution.
- Mix Binding Mix and MagJET Magnetic Beads before filling in the plate. In such case use 705 µL of this mixture per well.
- Transfer the NGS_Adapter_Duo protocol file to the KingFisher Duo instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.1 User Manual. The protocol files for MagJET Cleanup and Size Selection Kit can be found on product web page on www.thermoscientific.com/onebio
- Ensure that you are using the KingFisher Duo 12-pin magnet head and heating block.

1	Obtain one empty Thermo Scientific Microtiter 96 deep well plate, one Thermo Scientific KingFisher Duo elution strip and one Thermo Scientific KingFisher Duo 12-Tip strip.
2	Add 705 µL of **Binding Mix and MagJET Magnetic Beads mixture** to **row A**. **Note.** Resuspend magnetic bead suspension well before first use**.**
3	Add 100 µL of Elution Buffer to **row C** as indicated in Table 8, C1.
4	Add the following reagents to **rows D, E** and **Elution Strip** as described in Table 8. Note that **row B** is reserved for the tip and should be left empty. Note that rows **F-H** are left empty.
5	Transfer 100 µL DNA sample to row A with pre-mixed MagJET™ Magnetic Beads and Binding Mix.
6	Switch on the KingFisher Duo instrument and start the **NGS_Adapter_Duo** protocol. Insert the **NGS Adapter** plate and **Elution Strip** into the instrument as indicated on the KingFisher Duo display. Make sure that the **Elution Strip** is placed in the correct orientation into the elution block. Ensure that the perforated end is facing towards the user.

7	When the KingFisher Duo pauses at the **rebind** step (approximately 12 minutes after starting the run), remove the **NGS Adapter** plate from the instrument and add 705 µL of **Binding Mix and MagJET Magnetic Beads mixture** per well to **row C** to rebind the DNA (Table 8, C2).
8	Place the **NGS Adapter** plate back into the instrument and press OK. After the pause, the protocol will continue through to completion.
9	After the run is finished, remove the plates and turn off the instrument. Transfer the eluate from **Elution Strip** (containing DNA) to a new, clean vessel. The purified DNA is ready for use in downstream applications or can be stored at -20 °C for later use.

Table 8. Sample and reagent setup scheme.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
NGS Adapter Plate (Microtiter deep well 96 plate)	A	Sample	MagJET Magnetic Beads	5 µL
			Binding Mix	700 µL
			DNA sample	100 µL
	B	Tip	12-tip comb	*Empty*
	C1	Elution_1	Elution Buffer	100 µL
	C2	Rebind	MagJET Magnetic Beads	5 µL
			Binding Mix	700 µL
	D	Wash_1	Wash solution	400 µL
	E	Wash_2	Wash solution	400 µL
	F	*Empty*	*Empty*	*Empty*
	G	*Empty*	*Empty*	*Empty*
	H	*Empty*	*Empty*	*Empty*
KingFisher Duo Elution strip	-	Elution_2	Elution Buffer	50 µL

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Too much starting material was used.** Reduce the amount of starting material. Do not use more than 5 µg of DNA and make sure not to exceed 100 µL of starting sample volume. **Incomplete re-suspension of magnetic particles.** Always resuspend the magnetic particles by vortexing before use. **Loss of magnetic beads during purification.** Allow sufficient time to capture magnetic beads. Be careful not to remove any magnetic beads during procedures. Use smaller scale pipette tips. **Incorrect concentration or no isopropanol in Binding Mix.** Make sure that the isopropanol was properly mixed with Binding Buffer before each procedure. Follow the instructions for Binding Mix preparation on page 6. Only freshly prepared Binding mix can be used in the procedure. Using Binding Mix older than 24 hours results in decreased yield. **Incorrect concentration or no ethanol in Wash Buffer.** Ensure that ethanol was added to Wash Buffer before the first use. Follow the instructions for Wash Buffer preparation on page 6. **Too short binding time.** Make sure that DNA sample was completely mixed with magnetic beads and Binding Mix and incubated for at least 5 minutes during purification. **Elution Volume**. There should be an adequate volume of the Elution Buffer to cover the MagJET Magnetic Beads completely during the elution step.
Adapter residues	**Too many adapters used.** The amount of sequencing adapters in the ligation reaction exceeded 7 µg. Re-purify using Protocol A again to remove excess adapters. **Supernatant not completely removed.** Ensure all of the supernatant is removed after initial binding step.
Purified DNA is outside the range of interest	**Incorrect concentration or no isopropanol in Binding Mix.** Make sure that the isopropanol was properly mixed with Binding Buffer before procedures. Follow the instructions for Binding Mix preparation on page 6. Only fresh-prepared Binding Mix can be used in the procedure. Using Binding Mix older than 24 hours tends to disrupt binding conditions. **Incorrect binding conditions.** Size selection protocol is extremely sensitive to ratios of Binding Mix used. Choose the ratios from the **Calibration protocol** and make sure to add correct volumes of Binding Mix **Order of component mixing.** Make sure to mix all of the components in the order described. **Insufficiently fragmented DNA library.** This kit is **not** suited for fragment lengths above 1,000 bp. **Faulty pipettes.** Make sure to use well-calibrated pipettes. Also, to obtain reproducible size selection results, new pipette tips should be used for each sample when Binding Mix is added.

Problem	Possible cause and solution
Suboptimal A260/A280 ratio from UV measurement	**Magnetic particles are left in the elution.** Centrifuge eluate at full speed for 1 minute, place it in the magnetic rack for 2-3 min and transfer cleared supernatant to a new tube or plate.
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual salt.** Insufficient washing causes impurities in the Elution step. Ensure that correct volumes of the Wash Buffer are added as indicated in the protocol. **Purified DNA contains residual ethanol.** It is important to dry magnetic beads before elution step.
Carryover of the magnetic beads in the elution	**Carryover of the MagJET Magnetic Beads to the Elution step** may affect the A260/A280 ratio; however the magnetic beads in the eluted DNA will not affect downstream applications. To remove carryover magnetic particles place eluted sample in the magnetic rack once again. Carefully transfer eluate to a clean, sterile microcentrifuge tube.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.