invitrogen

SureCast™ Handcast System

For preparation of handcast mini gels for use with the Mini Gel Tank and XCell *Surelock*™ systems

USER GUIDE

Catalog Numbers HC1000, HC1000S, and HC1000SR

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Contents

Product description

The Invitrogen™ SureCast™ Handcast Station is used to cast your own polyacrylamide gels. Instructions are provided for casting gels using SureCast™ reagents, but reagents can be substituted with equivalent materials if following alternative recipies for resolving gel and stacking gel solutions.

Gels prepared with this protocol can be used in the XCell *SureLock*™ Mini-Cell or Mini Gel Tank. Use buffers and run conditions designated for Novex Tris-glycine gels.

Kit contents

The components included with the SureCast™ Handcast System (Cat. no. HC1000) are listed below. See "Description of parts" (page 4) for more details.

Components	Catalog Number	Storage
SureCast™ Gel Handcast Station	HC1000	Room temperature
SureCast™ Glass Plates	HC1001	
SureCast™ Sealing Pads	HC1002	
SureCast™ 10-well Multi-Use Tool	HC1010	
SureCast™ 12-well Multi-Use Tool	HC1012	
SureCast™ 15-well Multi-Use Tool	HC1015	
SureCast™ Gel Spacer	HC1003	
SureCast™ Gel Combs, variety pack	HC1005	
Reagents		
SureCast™ Stacking Buffer (1L), 2-pack	HC2112	Room temperature
SureCast™ Stacking Buffer (2.5L), 5-pack	HC2115	
SureCast™ Resolving Buffer (1L), 2-pack	HC2212	
SureCast™ Resolving Buffer (2.5L), 5-pack	HC2215	
SureCast™ APS	HC2005	
SureCast™ Acrylamide Solution - 40%	HC2040	
SureCast™ TEMED	HC2006	

Description of parts

Introduction

The parts of the SureCast™ Handcast System are described in the following section. See page 14 for ordering information.

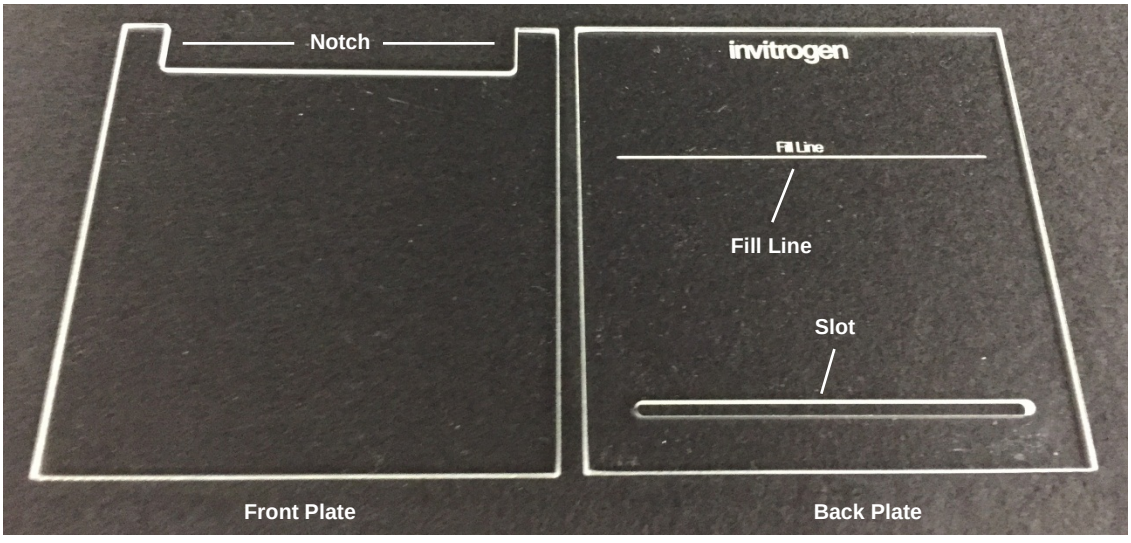
SureCast™ Handcast Station

The SureCast™ Handcast Station consists of a plastic base in which glass plates and a gel spacer are held in position with a cam plate to allow casting of polyacrylamide gels.

SureCast™ Glass Plates

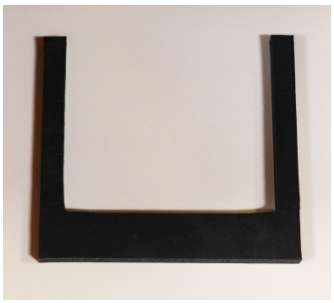
SureCast™ Glass Plates consist of a front plate and a back plate. The front plate has a notch at the top of the plate for placement of the gel comb when casting gels.

The back plate has a slot at the bottom, and a printed fill line to indicate the level to which resolving gel solution is poured when casting gels. When assembled, the lettering on the back plate should be readable, indicating that the plate is in the correct orientation.



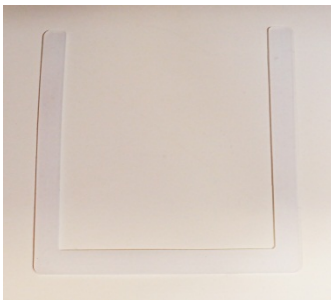
SureCast™ Sealing Pad

The SureCast™ Sealing Pad is attached to the cam plate, and ensures that the slot in the back plate is sealed against leakage when pouring the resolving gel. The pad also holds the glass plates firmly in place for casting.



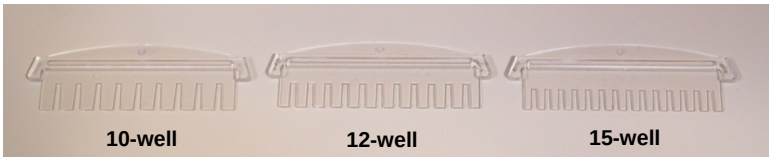
SureCast™ Gel Spacer

The SureCast™ Gel Spacer is a 1 mm thick silicone spacer that is placed between front and back SureCast™ Glass Plates when casting gels. The spacer is matched with the SureCast™ gel combs to cast 1 mm thick gels.



SureCast™ Gel Combs

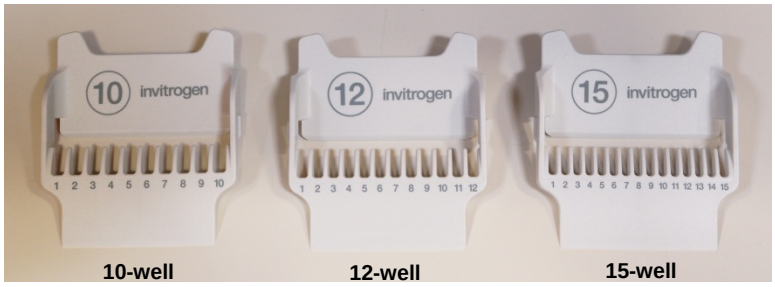
The SureCast™ Gel Combs are plastic combs with 1 mm thick teeth, used to cast 10-well, 12-well, and 15-well gels.



SureCast™ Multi-use Tool

The SureCast™ Multi-use Tool can be used for several purposes:

- Gel loading guide for 10-well, 12-well, or 15-well gels
- Gel cassette opening tool
- Gel cutting tool
- Gel spatula



Reagents

The SureCast™ solution are provided to pour high quality gels, and can be stored at room temperature.

- SureCast™ Resolving Buffer
- SureCast™ Stacking Buffer
- SureCast™ Acrylamide Solution – 40%
- SureCast™ APS
- SureCast™ TEMED

Product specifications

SureCast™ Handcast Station specifications

Dimensions:	10 × 10 × 10 cm
Material:	Plastic
Glass Plate Size:	10 cm × 10.5 cm

The SureCast™ Handcast Station is compatible with alcohols, but not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene) or acetone.

Gel selection

General guidelines

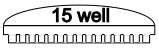
Choose the polyacrylamide percentage and type of well for your application based upon the expected molecular weight of your protein of interest and the volume of your sample.

Resolve large molecules with low percentage gels and small molecules with high percentage gels. If the molecular weight of the molecule is unknown, or the sample contains a wide range of molecules, use a mid-range percentage gel.

Recommended loading volumes

The maximum recommended loading volumes and protein load per band are provided in the table below.

Note: To ensure the highest band resolution, do not exceed the maximum load volume.

Well Types	Gel Thickness	Maximum Recommended Volume	Maximum Protein Load Per Band*
	1.0 mm	25 µL	0.5 µg/band
	1.0 mm	20 µL	0.5 µg/band
	1.0 mm	15 µL	0.5 µg/band

* Protein load values are for purified proteins that are western blotted subsequent to electrophoresis. For direct Coomassie staining (e.g. SimplyBlue™ Safestain), protein loads should be increased 10-fold.

Before starting

Prepare resolving and stacking buffers

If required, prepare resolving and stacking buffer solutions according to the instructions printed on the packaging.

1. Dissolve the contents of one SureCast™ Resolving Buffer pack in deionized water to a final volume of 500 mL.
2. Dissolve the contents of one SureCast™ Stacking Buffer pack in deionized water to a final volume of 500 mL.

The reconstituted buffers can be stored at room temperature for at least 3 months.

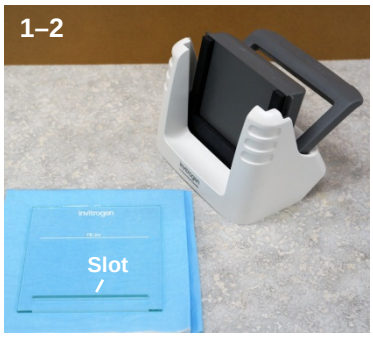
Prepare 10% APS (fresh)

To ensure proper polymerization of SureCast™ Gels, prepare fresh 10% APS before casting gels.

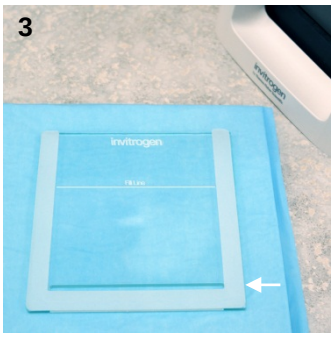
Dissolve 0.3 g of SureCast™ APS in 3 mL of deionized water.

Procedure

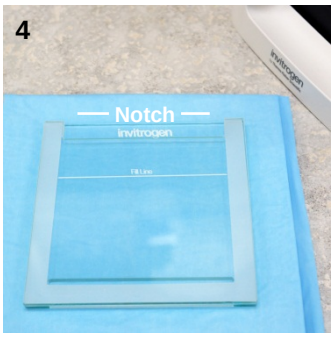
Set up the SureCast™ Handcast Station



1. Set the handcast station on a level surface.
2. Place the back glass plate on the benchtop so that the print is in the correct orientation for reading.



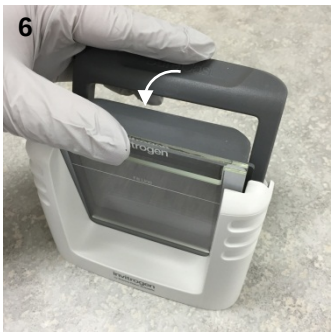
3. Place the silicone spacer on the back plate, and align it to the slot.



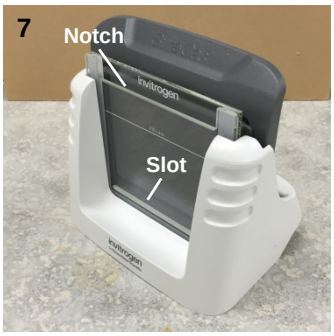
4. Place the front glass plate on the stack with the notch on top.

5. Hold the glass plate assembly so that all the outer edges are flush and place it in the handcast station.

   The bottom edge of the glass plate assembly should make contact with the base of the handcast station.



6. Hold the glass plate assembly to prevent shifting, and close the handle to secure the glass plates in the handcast station.



7. Verify that the parts are properly aligned before pouring the gel. Improper assembly can result in leakage of acrylamide solution.

Prepare resolving gel solution

WARNING: Before handling, read all applicable Safety Data Sheets (SDS) at **thermofisher.com/support**

1. Prepare resolving gel solution according to the volumes in the following table. The volumes provided in the table are for a single gel. Scale volumes proportionally based on the number of gels to be cast.

   Note: Solutions do not require degassing.

Solution	Polyacrylamide %								
	4%	6%	8%	10%	12%	14%	16%	18%	20%
SureCast™ Acrylamide (40%)	0.8 mL	1.2 mL	1.6 mL	2.0 mL	2.4 mL	2.8 mL	3.3 mL	3.6 mL	4.0 mL
SureCast™ Resolving Buffer	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL
Distilled water	5.1 mL	4.7 mL	4.3 mL	3.9 mL	3.5 mL	3.1 mL	2.7 mL	2.3 mL	1.9 mL
10% SureCast™ APS	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL
Total	**8 mL**	**8 mL**	**8 mL**	**8 mL**	**8 mL**	**8 mL**	**8 mL**	**8 mL**	**8 mL**

2. Add 8 µL of SureCast™ TEMED for every 8 mL of resolving gel solution. Mix well (but gently) and proceed immediately to "**Pour resolving gel**", page 9.

Pour resolving gel

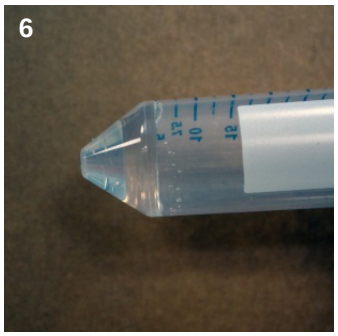
1. Tilt the handcast station to recline on the heel.

2. Add resolving gel solution to the gap between the glass plates until the solution reaches the level of the fill line.

3. (*Optional*) Carefully overlay the resolving gel solution with 0.5–1.0 mL of water-saturated 2-butanol, degassed water, or isopropanol.

4. Set the handcast station back to the upright position.

5. Allow the resolving gel to polymerize (5–10 min). The interface becomes more distinct as the gel polymerizes.



6. Verify polymerization by examining left over acrylamide in the tube.

7. If overlay was used, pour off the overlay solution and rinse with water (dispose of butanol waste in the appropriate manner for organic chemicals).

8. Wick out any remaining liquid with a piece of blotting paper, and proceed to "**Prepare stacking gel solution**", page 10.

Prepare stacking gel solution

1. Prepare stacking gel solution according to the following table. The volumes provided in the table are for a single gel. Scale volumes proportionally based on the number of gels to be cast.

   Note: Solutions do not require degassing.

Solution	4%
SureCast™ Acrylamide (40%)	0.30 mL
SureCast™ Stacking Buffer	0.75 mL
Distilled water	1.92 mL
10% SureCast™ APS	30 µL
Total	**3 mL**

2. Add 3 µL of SureCast™ TEMED for every 3 mL of resolving gel solution. Mix well and proceed immediately to "**Pour stacking gel**".

Pour stacking gel

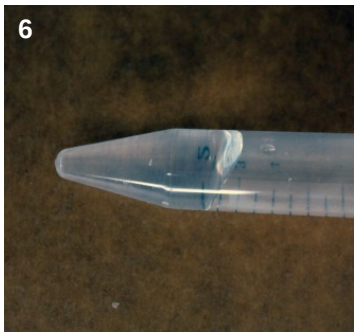
1. Tilt the handcast station to recline on the heel.

2. Add the stacking gel solution until it reaches the upper edge of the front glass plate.

3. Insert the comb slowly by starting at one end and sliding it between the glass plates until both ends are in place.

4. Set the handcast station back to the upright position.

5. Allow the stacking gel to polymerize (~10 minutes).



6. Verify polymerization by examining left over acrylamide in the tube.

Remove plate from casting station

Release the clamp and remove the glass plate assembly containing the polymerized gel. The gel can be used immediately, or wrapped in a damp paper towel and stored at 4°C for future use.

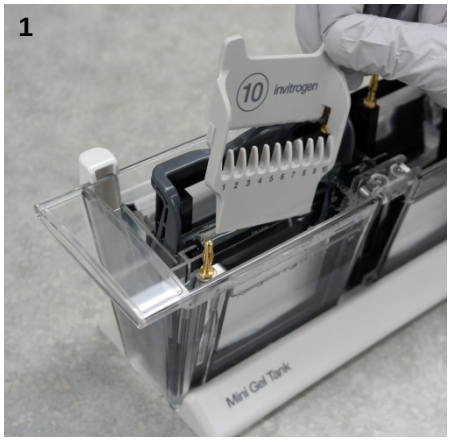
Gel electrophoresis

Insert SureCast™ Gel

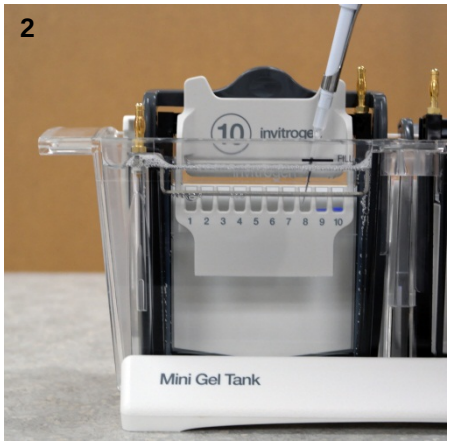
1. Use a pipette to gently wash the wells with 1X running buffer. Invert the gel and shake to remove buffer. Repeat twice. Fill the sample wells with running buffer.
2. Place the SureCast™ Gel into a XCell SureLock™ Mini-Cell or Mini Gel Tank according to the instructions provided in their respective manuals.

Load wells

Use the Multi-use Tool to visualize the location of the wells when loading samples.



1. Slide the Multi-use Tool onto the glass plate.



2. Load samples and markers into wells.

Run conditions

Gels prepared with this protocol can be used in the XCell SureLock™ Mini-Cell or Mini Gel Tank. Use buffers and run conditions designated for Novex Tris-glycine gels.

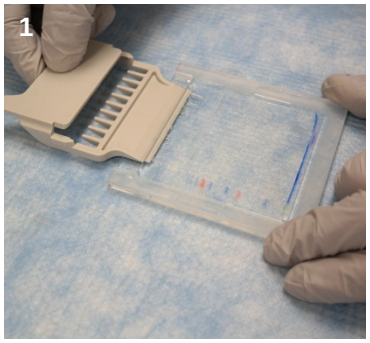
Run gels as described in the following table:

Running Buffer	Constant Voltage	Run Time*
Tris-Glycine Running Buffer	125 V	80–140 min

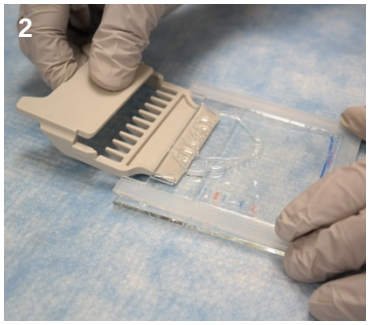
* Run time varies depending upon the percentage of the gel, the type of gel tank, and the power supply used for electrophoresis.

Removing the gel from the glass plate assembly

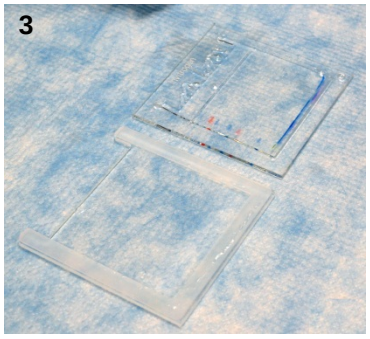
Open the glass plate assembly



1. Slide the Multi-use Tool into the gap between the front and back glass plates.

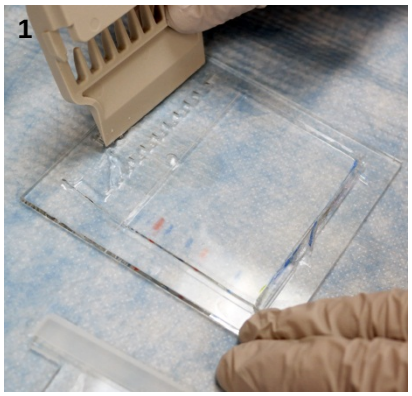


2. Use the Multi-use Tool to pry the glass plates apart.



3. Separate the two pieces of glass.

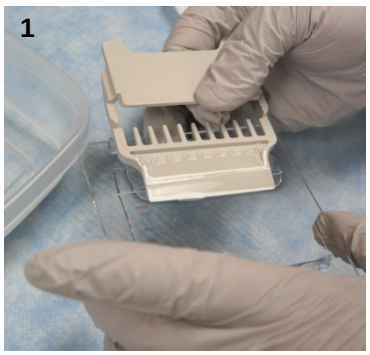
Remove the wells and foot of the gel



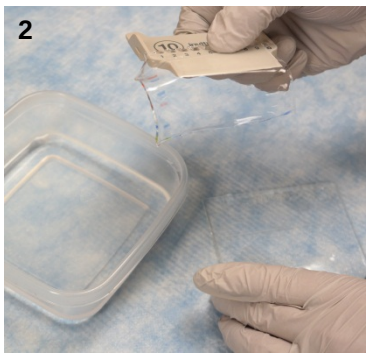
1. Use the blade of the Multi-use Tool to cut off the portions of the gel that form the wells.

2. Use the blade of the Multi-use Tool to cut off the portion of gel in the slot. Make the cut just above the slot.
Alternately, see "**Remove the gel from the glass plate**", page 13.

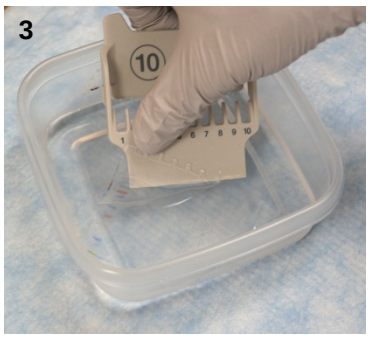
Remove the gel from the front plate using the Multi-use Tool



1. Slide the Multi-use Tool under the gel, and gently separate the gel from the glass plate.

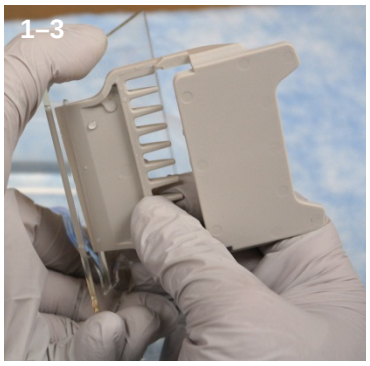


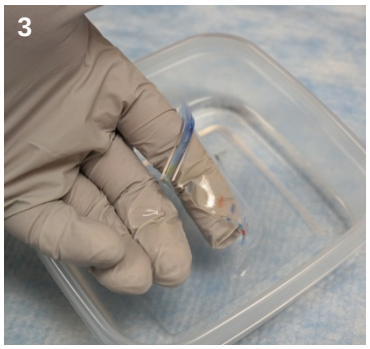
2. Carefully lift the gel from the glass plate, while holding one edge against the Multi-use Tool to prevent the gel from sliding off.



3. Transfer the gel into a tray of water.
4. Proceed to gel staining or other downstream application (e.g., western transfer).

(*Optional*) Remove the gel from the glass plate





1. Wet your glove with water to prevent the gel from sticking to the glove.
2. Hold the glass plate over a tray containing water.
3. Insert the blade of the Multi-use Tool through the slot, and push gently to release the gel from the glass plate.
4. Allow the gel to slide from the glass plate, and onto your hand.
5. Lower the gel into the tray of water.
6. Proceed to gel staining or other downstream application (e.g. western transfer).

Maintenance

General guidelines

- After casting gels, dispose of the waste materials in an appropriate manner.
  - Polymerized polyacrylamide is not considered hazardous and can be disposed of in regular trash.
  - Waste butanol used for overlaying the resolving gel, must be disposed of as an organic solvent.
- Rinse and wipe the handcast station to remove any residual liquids and solidified polyacrylamide.
- Clean the surface of the glass plates by rinsing and rubbing gently under a water stream. **Do not** use harsh detergents or solvents. Use the blade of the Multi-use Tool to scrape out any residual gel material stuck in the slot of the back plate.
- Rinse and wipe the Multi-use Tool to remove any residual liquids and residual gel material.
- SureCast™ Sealing Pads may wear out and need to be replaced. See page 15 for ordering information.

Appendix A

Troubleshooting

The following table provides some solutions to possible problems that may be encountered when casting polyacrylamide gels.

Observation	Cause	Solution
Resolving or stacking gel polymerizes too quickly	Improper addition of TEMED	Make sure that TEMED is added to resolving or stacking gel solutions just before pouring.
	Incorrect amount of APS used in gel solution	Make sure that 10% APS is used to prepare gel solutions
Resolving or stacking gel does not polymerize	Problem with 10% APS solution	Ensure that fresh 10% APS is used to prepare resolving and stacking gel solutions.
	TEMED not added to resolving or stacking gel solution	Make sure that TEMED is added to resolving or stacking gel solution just before pouring.
Resolving gel solution leaking from glass plate assembly	Glass plate assembly not properly seated in handcast station	Ensure that glass plates and silicone spacer are properly aligned and placed in the handcast station with the bottom edge of the glass plate assembly in contact with the base of the handcast station (page 7).
	Damage to silicone spacer	Replace damaged spacer. See page 15 for ordering information.
	Sealing pad on cam plate worn out	Replace worn out sealing pad. See page 15 for ordering information.
	Residual gel material stuck on glass plates, spacer, or handcast station	Clean plates, spacer, and handcast station after every use. See page 13 for maintenance guidelines.
Irregular surface between resolving gel and stacking gel	Solution not level in glass plate assembly after being poured	Rock the handcast stand side-to-side after adding resolving gel solution until the surface becomes level.
		Add overlay solution on top of resolving gel solution (page 9).
Wells not properly formed	Insufficient stacking gel solution added to glass plate assembly	Ensure that stacking gel solution is filled to the upper edge of the front glass plate.
	Bubbles trapped between teeth of gel combs	Make sure that bubbles do not become trapped between the teeth of the gel comb during insertion.

Appendix B

Related products

Additional products

Many of the components of the SureCast™ Handcast System, as well as additional reagents that may be used for electrophoresis of proteins are available separately from ThermoFisher Scientific. Ordering information is provided below. For details, visit **thermofisher.com** or call Technical Support (page 15).

Apparatus	Quantity	Cat. no.
Mini Gel Tank	1 unit	A25977
XCell SureLock™ Mini-Cell	1 unit	EI0001
PowerEase™ 90W Power Supply	1	PS0090 PS0091 PS0092
PowerEase™ 300W Power Supply	1	PS0300 PS0301 PS0302
Replacement Parts	**Quantity**	**Cat. no.**
SureCast™ Glass Plates	2 pair	HC1001
SureCast™ Sealing Pads	2 pads	HC1002
SureCast™ Gel Spacers	10 spacers	HC1003
SureCast™ 10-well Multi-Use Tool	1	HC1010
SureCast™ 12-well Multi-Use Tool	1	HC1012
SureCast™ 15-well Multi-Use Tool	1	HC1015
Gel Runner Tank	1	B4478641
Cassette Clamp (Left)	1	B4478593
Cassette Clamp (Right)	1	B4478592
Mini Gel Tank Lid	1	B4478591
Mini Gel Tank Base	1	B4478640
Empty Gel Cassette Combs, mini, 1.0 mm, 10 well	25 combs	NC3010
Empty Gel Cassette Combs, mini, 1.0 mm, 12 well	25 combs	NC3012
Empty Gel Cassette Combs, mini, 1.0 mm, 15 well	25 combs	NC3015
Novex™ Power Supply Adapters	1 set	ZA10001
Pipette Tips for Gel Loading	**Quantity**	**Cat. no.**
Gel Loading Tips (Standard Round)	200/pk	LC1001
Flat Gel Loading Tips	200/pk	LC1002
Gel Loading Tips (Eppendorf Round)	200/pk	LC1010
Pre-Mixed Buffers	**Quantity**	**Cat. no.**
Novex™ Tris-Glycine SDS Sample Buffer (2X)	20 mL	LC2676
NuPAGE™ Sample Reducing Agent (10X)	250 µL	NP0004
	10 mL	NP0009
Novex™ Tris-Glycine SDS Running Buffer (10X)	500 mL	LC2675
	4 × 1 L	LC2675-4
	5 L	LC2675-5

Technical support

Obtaining support

For the latest services and support information for all locations, go to **thermofisher.com**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **thermofisher.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited product warranty

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