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SureCast™ Handcast System

Pub. No. MAN0014074 Rev A.0

Product description

The 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 recipes 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.

Required reagents

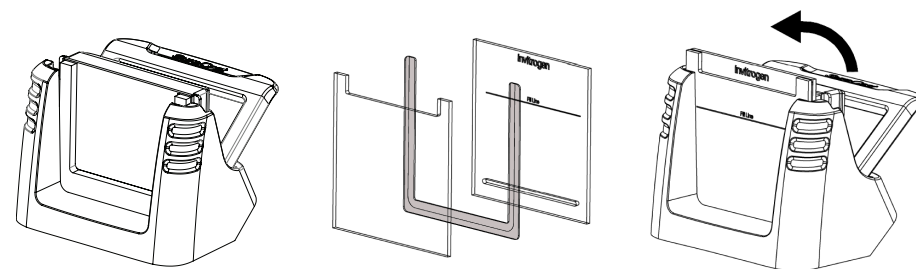
The following reagents (or their equivalent) are required for preparing solutions to cast SureCast™ gels.

- SureCast™ Resolving Buffer
- SureCast™ APS
- SureCast™ Stacking Buffer
- SureCast™ TEMED
- SureCast™ Acrylamide

Set up SureCast™ Handcast Station

Cast gels according to guidelines in the product Safety Data Sheets (SDS) and the EHS rules of your institution.

1. Set the handcast station on a level surface.
2. Assemble the glass plates with the silicone spacer between them so that all the outer edges are flush.
3. Place the glass plate assembly in the handcast station.
4. Hold the glass plate assembly to prevent shifting, and close the handle to secure the glass plates in the handcast station.



Prepare 10% APS (fresh)

Dissolve 300 mg of SureCast™ APS in 3 mL of deionized water.

Prepare resolving gel solution

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

1. Prepare resolving 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:** Solution does not require degassing.

Solution	Polyacrylamide %			
	4%	10%	12%	20%
SureCast™ Acrylamide (40%)	0.8 mL	2.0 mL	2.4 mL	4.0 mL
SureCast™ Resolving Buffer	2.0 mL	2.0 mL	2.0 mL	2.0 mL
Distilled water	5.1 mL	3.9 mL	3.5 mL	1.9 mL
10% SureCast™ APS	80 µL	80 µL	80 µL	80 µL
Total	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**.

Pour resolving gel



Heel



1. Tilt the handcast station to recline on the heel.
2. Add resolving gel solution to the glass plates until the solution reaches the level of the fill line.
3. (Optional) Carefully overlay the resolving gel solution with butanol, 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 waste in the appropriate manner). Wick out any remaining liquid with a piece of blotting paper, and proceed to **Prepare stacking gel solution**.

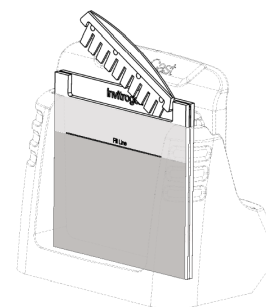
Prepare stacking gel solution

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

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. Set the handcast station back to the upright position.
4. Insert the comb slowly by starting at one end and sliding it between the glass plates until both ends are in place.
5. Allow the stacking gel to polymerize (5–10 min).
6. Verify polymerization by examining left over acrylamide in the tube.
7. Release the clamp and remove the plate. The gel can be used immediately, or wrapped in a damp paper towel and stored at 4°C for future use.