QUICK REFERENCE

novex by life technologies

GEL CASTING INSTRUCTIONS

Cat. nos. NC2010, NC2015, NW2010

Doc. Part no. 30909.INS **Pub. no.** MAN0001660 **Rev.** A.0

WARNING! Before handling, read all applicable Safety Data Sheets (SDSs) at **www.lifetechnologies.com/support**.

Before starting

Prepare the following stock solutions:

- **50% Acrylamide/BIS (29:1)**
  48.3 g Acrylamide
  1.7 g BIS
  Bring to 100 mL with water
- **10% SDS**
  10.0 g SDS
  Bring to 100 mL with water
- **Catalyst (Make fresh on the day of use)**
  100 mg Ammonium Persulfate in 2 mL of water
- **Separating Gel Buffer (1 M Tris-HCl, pH 8.8)**
  Add 30.3 g Tris to about 150 mL water; adjust to pH 8.8 with HCl
  Bring to 250 mL with water
- **Stacking Gel Buffer (0.375 M Tris HCl, pH 6.8)**
  Add 11.4 g Tris to about 150 mL water; adjust to pH 6.8 with HCl
  Bring to 250 mL with water
- **50% Sucrose**
  50.0 g Sucrose
  Bring to 100 mL with water

Notes

- Store all of the solutions at room temperature.
- Store 50% Acrylamide/BIS for up to two (2) months in a dark glass bottle.
- You may store the solutions (except for Catalyst) indefinitely as long as they remain free of particulates or microbial growth.
- Storage at room temperature eliminates the need to degas the solution before use.

Separating Gel

The following recipes are for approximately 25 mL of Separating Gel, enough for four 1.0-mm thick mini gels.

Solution	6% Gel Amount	8% Gel Amount	10% Gel Amount	12% Gel Amount
50% Acrylamide/BIS	3.0 mL	4.0 mL	5.0 mL	6.0 mL
Separating Gel Buffer	9.4 mL	9.4 mL	9.4 mL	9.4 mL
10% SDS	250 µL	250 µL	250 µL	250 µL
50% Sucrose*	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Water	7.8 mL	6.8 mL	5.8 mL	4.8 mL
TEMED**	6.25 µL	6.25 µL	6.25 µL	6.25 µL
Catalyst**	625 µL	625 µL	625 µL	625 µL

Solution	14% Gel Amount	16% Gel Amount	18% Gel Amount	20% Gel Amount
50% Acrylamide/BIS	7.0 mL	8.0 mL	9.0 mL	10.0 mL
Separating Gel Buffer	9.4 mL	9.4 mL	9.4 mL	9.4 mL
10% SDS	250 µL	250 µL	250 µL	250 µL
50% Sucrose*	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Water	3.7 mL	2.7 mL	1.7 mL	750 µL
TEMED**	6.25 µL	6.25 µL	6.25 µL	6.25 µL
Catalyst**	625 µL	625 µL	625 µL	625 µL

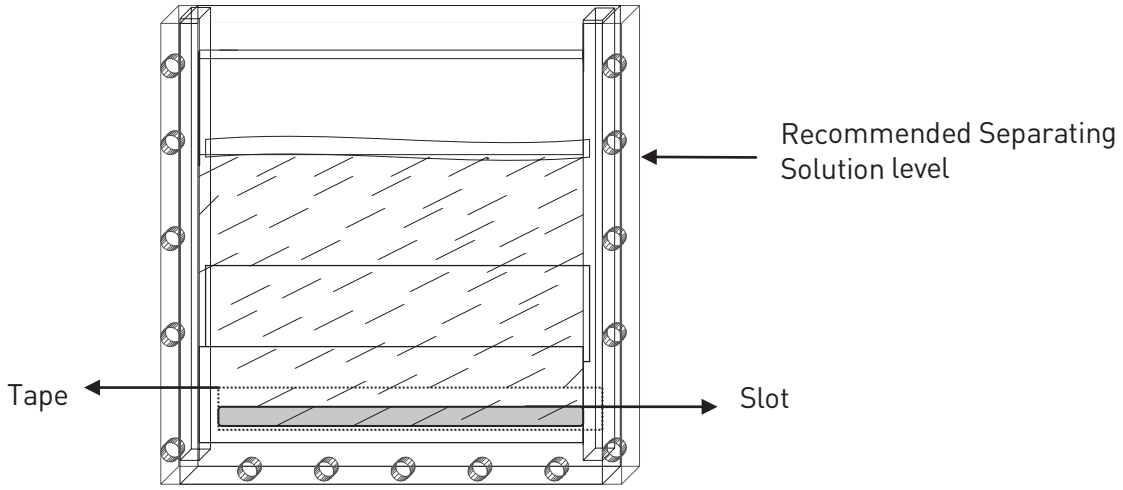
*Optional, but recommended because it makes it easy to form a good interface between the separating gel and the overlay. If omitted, increase the amount of water added to make up for the volume of the sucrose solution (increase the water by 4.0 mL for the above tables).

**Add these last and mix well just before the gel is to be poured.

life technologies

Gel Casting

1. Check that the tape at the bottom of the cassette is completely covering the slot.
2. Set the cassette in a suitable rack so that it is in a vertical position.
3. Immediately after the separating gel solution has been prepared and mixed, fill the cassette to just above the horizontal line located about 2 cm from the top edge of the U-shaped plate.
4. Check that there are no air bubbles trapped in the slot at the bottom of the cassette.
5. Using a transfer pipette, carefully overlay the separating gel with degassed water.
6. Continue adding water until the cassette is filled.
7. Allow the separating gel to polymerize for about 1 hour. The interface will become more distinct as the gel polymerizes.



Stacking Gel

4% Stacking Gel (12.5 mL)

Solution	Amount
50% Acrylamide/BIS	1.0 mL
Stacking Gel Buffer	4.2 mL
10% SDS	125 µL
Water	6.3 mL
TEMED**	5.0 µL
Catalyst**	1.0 mL

** Add these just before the Stacking Gel is to be poured.

1. Prepare the Stacking Gel Solution (see the **4% Stacking Gel Table**) and mix well.
2. Pour out the overlay solution; allow the solution to drain for a few seconds so that it is completely removed.
3. Fill the cassette to within a couple millimeters of the top with the stacking gel solution.
4. Insert the comb by starting at one end and rocking it down until both ends are in place. Make sure the comb is completely inserted. Allow about 30 minutes to 1 hour for the stacking gel to polymerize completely.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

lifetechnologies.com

1 November 2014