invitrogen™

Novex® Midi Cassettes and Combs

Catalog no.	Comb Type	Quantity
WC1012	12 + 2 well	10/pack
WC1020	20 well	10/pack
WC1026	26 well	10/pack

Introduction

Novex® Empty Midi Cassettes and Combs are disposable plastic cassettes with combs that allow you to pour your own midi gels for use with the XCell4 *SureLock*™ Midi-Cell. The cassettes are sealed on three sides to prevent leaking and do not require the use of a special casting stand. The cassettes are designed for pouring 1.0 mm thick gels with a choice of three comb types (12 + 2 wells, 20 wells, and 26 wells) and have printed well outlines for easier gel loading.

Specifications

Cassette Size:	15 cm x 10.3 cm
Cassette Material:	Styrene Copolymer
Gel Thickness:	1.0 mm
Comb Types:	12+2, 20, and 26 well

Caution

Acrylamide is known to the state of California to cause cancer. To obtain a MSDS, visit www.invitrogen.com/msds. Acrylamide is toxic if swallowed and is harmful in contact with skin. Always wear gloves, laboratory coat, and safety glasses when handling acrylamide and preparing gels.

Materials Needed

- Stock Solutions (Acrylamide/bis, Separating and Stacking gel Buffer, SDS, sucrose, and ammonium persulfate); see below for recipes
- UltraPure™ TEMED (Cat. no. 15524-010)
- Appropriate rack such as a test tube rack
- Degassed water

Preparing Stock Solutions

Prepare the following stock solutions as described below. Some of the solutions are available premade from Invitrogen or as dry reagents, see ordering information listed below.

50% Acrylamide/bis (29:1)

Acrylamide (Cat. no. 15512-023)	48.3 g
N,N'-Methylenebisacrylamide (Cat. no. 15516-024)	1.7 g
Deionized Water	to 100 mL

Mix, filter, and store at room temperature in the dark. Stable for up to 2 months when stored at room temperature in the dark.

Separating Gel Buffer (1 M Tris-HCl, pH 8.8)

Add 30.3 g Tris base to 150 mL deionized water. Mix and adjust the pH to 8.8 with 6 N HCl. Adjust the volume to 250 mL with deionized water and store at room temperature.

Stacking Gel Buffer (0.375 M Tris-HCl, pH 6.8)

Add 11.4 g Tris base to 150 mL deionized water. Mix and adjust the pH to 6.8 with 6 N HCl. Adjust the volume to 250 mL with deionized water and store at room temperature.

10% SDS (Cat. no. 15553-027)

Add 10 g SDS to 90 mL deionized water. Mix slowly until SDS is dissolved. Adjust the volume to 100 mL with deionized water and store at room temperature.

10% Ammonium Persulfate (prepare fresh before use)

Add 100 mg ammonium persulfate (APS) to 2 mL deionized water. Mix well and use immediately.

50% Sucrose

Add 50 g sucrose (Cat. no. 15503-022) to 90 mL deionized water. Mix well until sucrose is dissolved. Adjust the volume to 100 mL with deionized water and store at room temperature.

Note: Stock solutions stored at room temperature **do not** need to be degased before use. All stock solutions are stable indefinitely, except acrylamide/bis, as long as they are free of particulate material or microbial growth.

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Casting the Separating Gel

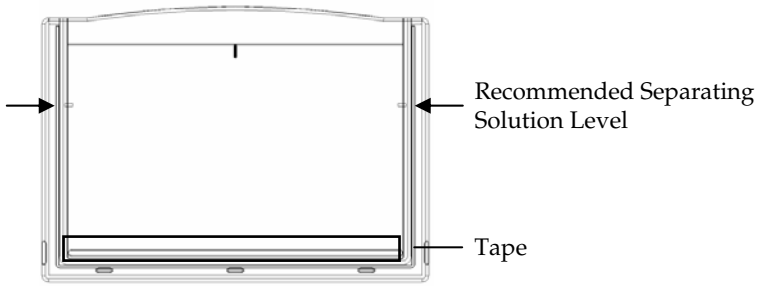
1. Remove the Novex® Empty Midi Cassette and Comb from the box and ensure the tape at the bottom is completely covering the slot.
2. Remove comb from the cassette and place the cassette in a vertical position in a suitable rack (such as a tube rack).
3. Prepare and mix 50 mL Separating Gel Solution using the recipe given below to cast four 1.0 mm thick midi gels of the desired acrylamide/bis percentage:

Solution	**6%**	**8%**	**10%**	**12%**	**14%**	**16%**	**18%**	**20%**
50% Acrylamide/bis (29:1)	6.0 mL	8.0 mL	10.0 mL	12.0 mL	14.0 mL	16.0 mL	18.0 mL	20 mL
Separating Gel Buffer	18.8 mL	18.8 mL	18.8 mL	18.8 mL	18.8 mL	18.8 mL	18.8 mL	18.8 mL
10% SDS	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL
50% Sucrose* (optional)	8.0 mL	8.0 mL	8.0 mL	8.0 mL	8.0 mL	8.0 mL	8.0 mL	8.0 mL
Deionized water	15.6 mL	13.6 mL	11.6 mL	9.6 mL	7.4 mL	5.4 mL	3.4 mL	1.4 mL
TEMED**	12.5 µL	12.5 µL	12.5 µL	12.5 µL	12.5 µL	12.5 µL	12.5 µL	12.5 µL
10% APS** **(fresh)**	1.25 mL	1.25 mL	1.25 mL	1.25 mL	1.25 mL	1.25 mL	1.25 mL	1.25 mL

*The addition of 50% sucrose solution is optional but is recommended as the solution helps in the formation of a good interface between the separating gel and the overlay. If you do not wish to use the sucrose solution, use deionized water to make up the volume of the sucrose solution (use 4.0 mL deionized water in the above table).

Add the TEMED and 10% APS **last to the solution and mix well just prior to pouring the solution.

4. Immediately after preparing and mixing the Separating Gel Solution, pour the solution into the empty cassette without generating air bubbles. Fill the cassette up to the **Recommended Separating Solution Level** shown in the figure below or up to the alignment slots on the cassette (indicated with the arrows on the figure).



5. Using a transfer pipette, carefully overlay the separating gel with degassed water and continue adding to fill the cassette with degassed water.
6. Allow the separating gel to polymerize for about 1 hour. The interface becomes more distinct as the gel polymerizes.

Casting the Stacking Gel

1. Prepare and mix 12.5 mL 4% Stacking Gel Solution using the recipe given below to cast four 1.0 mm thick midi gels:

Solution	**4% Stacking Gel**
50% Acrylamide/bis (29:1)	1.0 mL
Stacking Gel Buffer	4.2 mL
10% SDS	125 µL
Deionized water	6.3 mL
TEMED*	5.0 µL
10% APS* **(fresh)**	1.0 mL

Add the TEMED and 10% APS **last to the solution and mix well just prior to pouring the solution.

2. Decant the overlay solution from the cassette and briefly rinse the separating gel with distilled water.
3. Immediately after preparing and mixing the Stacking Gel Solution, pour the solution into the empty cassette without generating air bubbles. Fill the cassette to within a few millimeters of the top of the cassette.
4. Insert the appropriate comb by starting at one end and rocking the comb down slowly until both ends of the comb are in place. Ensure the comb is completely inserted and there are no air bubbles trapped between the comb and stacking gel.
5. Allow the stacking gel to polymerize for about 30 minutes to 1 hour.
6. Gently pull the comb out of the cassette, rinse the wells with 1X Running Buffer, and fill the sample wells with 1X Running Buffer. Use the gel with the XCell4 *SureLock*™ Midi-Cell as described in the Midi-Cell manual.