

Procedures for Gel Preparation with AcrylaGel™ and Bis-AcrylaGel™

National Diagnostics' AcrylaGel (EC-810) is a ready-to-use 30% acrylamide solution in distilled, deionized water. AcrylaGel can be cross-linked with Bis-AcrylaGel (EC-820), our ready-to-use, 2% solution of methylene bisacrylamide. Alternatively, any powdered acrylamide crosslinking reagent can be used with AcrylaGel. Store

solutions tightly capped in a dark area at room temperature (20°C). Acrylamide has been found to be neurotoxic. Protective eyewear and gloves should be worn while handling these products. If accidental exposure occurs, contact a physician immediately.

Mix Gel Solution

Calculate how much AcrylaGel and Bis-AcrylaGel you need to make your gels by using the formulas at right. Bring up to the desired final volume with your usual buffers and distilled water. Pour the solution into an Erlenmeyer flask with a side-arm. In most cases, AcrylaGel and Bis-AcrylaGel will gel without degassing. However, if degassing is desired, add a stirring bar to the solution and stopper the flask. Degas the solution under vacuum for 5 minutes while stirring on a magnetic stirrer.

$$V_a = \frac{(A)(V_t)}{30} \quad V_b = \frac{(A)(C)(V_t)}{200}$$

V_a = Volume of AcrylaGel to be used (ml),
 V_b = Volume of Bis-AcrylaGel to be used (ml),
 V_t = Total volume of gel casting solution desired (ml),
 A = % acrylamide desired in gel,
 C = % crosslinker desired = $\left(\frac{\text{g bis}}{\text{g acrylamide}} \times 100 \right)$

EXAMPLE: To make 100ml of a 10% acrylamide gel 2.7% crosslinked with bis, calculate the volume to be added as follows:

$$V_a = \frac{(10)(100)}{30} = 33.3\text{ml AcrylaGel}$$

$$V_b = \frac{(10)(2.7)(100)}{200} = 13.5\text{ml Bis-AcrylaGel}$$

Add APS and Cast Gel

Add 1.0ml of 10% (w/v) FRESHLY PREPARED 10% Ammonium Persulfate for every 100ml of gel casting solution. Swirl gently to mix. Add 0.1 ml of TEMED for every 100ml of gel casting solution. Swirl gently to mix. Pour the solution into the gel casting cassette. The gel should begin to set in 10-20 minutes. NOTE:

After two hours of polymerization wrap each end of the gel cassette with clear plastic wrap. This is important to keep the ends of the gel from drying and to maintain sample well integrity. Appropriately wrapped gels may be stored for up to 48 hours.

Suggestions for Best Results

- Clean glass plates thoroughly. Rinse with ethanol and wipe dry. Apply Glass Free™ (Cat. #EC-621) to one plate to ensure release after electrophoresis.
- Use fresh, highly qualified buffers and initiators.
- Degassing will ensure the reproducibility of results.

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