



# **Acrylamide and Bis-Acrylamide Solutions**

## **Instructions for Use**

**Catalog Number**

**161-0154 to 161-0159**

**161-0140 to 161-0149**

**BIO-RAD**

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# Section 1

## Acrylamide and Bis-Acrylamide Calculations

Use these calculations to determine the volumes of stock acrylamide and bis-acrylamide solutions necessary to produce gels of any percent and volume. Refer to Section 2 for predetermined formulas for 37.5:1 (2.67% C) and 19:1 (5% C).

### 1.1 To determine monomer and crosslinker ratios

- 1) (A) is the part of total monomer that is acrylamide

$$A = \frac{\text{g acrylamide}}{\text{g acrylamide} + \text{g bis-acrylamide}}$$

- 2) (C) is the part of total monomer that is bis-acrylamide

$$C = \frac{\text{g bis-acrylamide}}{\text{g bis-acrylamide} + \text{g acrylamide}}$$

### 1.2 To determine the volume of 40% acrylamide stock solution

- 1) Calculate the value (A) for the desired gel percentage (gel%) using the equation in Section 1.1.

- 2) Plug values into the equation

$$\frac{(A)(\text{gel})(\text{Final vol})}{(40)\% \text{ acrylamide solution}} = \text{ml of 40\% acrylamide}$$

### 1.3 To determine the volume of 2% bis-acrylamide stock solution

- 1) Calculate (C) for the desired gel percentage (gel%) from the equation in Section 1.1.
- 2) Plug values into equation:

$$\frac{(C)(\text{gel})(\text{Final vol})}{(2)\% \text{ bis-acrylamide solution}} = \text{ml of 2\% bis-acrylamide}$$

## Section 2

### Protocols for 40% Acrylamide and 2% Bis-Acrylamide Solutions

#### 2.1 Laemmli<sup>1</sup> separating gel, 37.5:1 ratio

	<b>Stack</b>	<b>Resolving Gel</b>		
	<b>4%</b>	<b>7.5%</b>	<b>12%</b>	<b>X%</b>
40% Acrylamide	2.4 ml	18.2 ml	29.2 ml	2.43(X%) = A* ml
2% Bis-Acrylamide	1.3 ml	10 ml	16 ml	1.34(X%) = B* ml
0.5 M Tris-HCl, pH 6.8	6.3 ml	–	–	–
1.5 M Tris-HCl, pH 8.8	–	25 ml	25 ml	25 ml
10% SDS	250 µl	1.0 ml	1.0 ml	1.0 ml
Distilled deionized water	14.6 ml	45.3 ml	28.3 ml	73.5 - (A+B)*
TEMED	25 µl	50 µl	50 µl	50 µl
10% Ammonium persulfate (APS)	<u>125 ml</u>	<u>500 ml</u>	<u>500 ml</u>	<u>500 ml</u>
Total volume	25 ml	100 ml	100 ml	100 ml

Degas before polymerization.

- \* The letter A designates the volume of 40% acrylamide solution required to produce the specified percent gel (X%). The letter B designates the volume of 2% bis-acrylamide solution required to produce a 37.5:1 crosslinker ratio in the specified percent gel (X%).

## 2.2 Sequencing gel<sup>2</sup>, 19:1 ratio

	<u>4%</u>	<u>6%</u>	<u>X%</u>
40% Acrylamide solution	14.3 ml	21.4 ml	$3.57(X\%) = A_a$ ml
2% Bis solution	15 ml	22.5 ml	$3.75(X\%) = B_b$ ml
10x TBE	15 ml	15 ml	15 ml
Urea	63 g	63 g	63 g
TEMED	150 $\mu$ l	150 $\mu$ l	150 $\mu$ l
25% APS	150 $\mu$ l	150 $\mu$ l	150 $\mu$ l

Adjust the volume to 150 ml with deionized distilled water. Degas before polymerization.

- The letter A designates the volume of 40% acrylamide solution required to produce the specified percent gel (X%).
- The letter B designates the volume of 2% bis-acrylamide solution required to produce a 19:1 crosslinker ratio in the specified percent gel (X%).

## Section 3

### Protocols for 40% Acrylamide/Bis Solutions, 19:1, 29:1, and 37.5:1

#### 3.1 Laemmli<sup>1</sup> resolving gel 29:1 or 37.5:1 ratio

	<u>Stack</u>	<u>Resolving Gel</u>		
	<u>4%</u>	<u>7.5%</u>	<u>12%</u>	<u>X%</u>
40% Acrylamide/Bis	2.5 ml	18.8 ml	30 ml	$2.5(X\%) = (A)^*$ ml
0.5 M Tris-HCl, pH 6.8	6.3 ml	–	–	–
1.5 M Tris-HCl, pH 8.8	–	25 ml	25 ml	25 ml
10% SDS	250 $\mu$ l	1.0 ml	1.0 ml	1.0 ml
Distilled deionized water	15.9 ml	54.7 ml	43.5 ml	$73.5 - (A)^*$
TEMED	25 $\mu$ l	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
10% APS	<u>125 <math>\mu</math>l</u>	<u>500 <math>\mu</math>l</u>	<u>500 <math>\mu</math>l</u>	<u>500 <math>\mu</math>l</u>
Total volume	25 ml	100 ml	100 ml	100 ml

Degas before polymerization.

- \* The letter A designates the volume of 40% Acrylamide/Bis Solution required to produce the specified percent gel (X%).

### 3.2 Sequencing gel ,19:1 ratio

	<u>4%</u>	<u>6%</u>	<u>X%</u>
40% Acrylamide/Bis 19:1	15 ml	22.5 ml	3.75(X%) = ml
10x TBE	15 ml	15 ml	15 ml
Urea	63 g	63 g	63 g
TEMED	150 $\mu$ l	150 $\mu$ l	150 $\mu$ l
25% APS	150 $\mu$ l	150 $\mu$ l	150 $\mu$ l

Adjust the volume to 150 ml with deionized distilled water.

Degas before polymerization.

### Section 4

### Protocols for 30% Acrylamide/Bis Solutions, 19:1, 29:1 and 37.5:1

#### 4.1 Laemmli<sup>2</sup> resolving gel, 29:1 or 37.5:1 ratio

	<u>Stack</u>	<u>Resolving Gel</u>		
	<u>4%</u>	<u>7.5%</u>	<u>12%</u>	<u>X%</u>
30% Acrylamide/Bis	3.3 ml	25 ml	40 ml	3.3(X%) = (A)* ml
0.5 M Tris-HCl, pH 6.8	6.3 ml	–	–	–
1.5 M Tris-HCl, pH 8.8	–	25 ml	25 ml	25 ml
10% SDS	250 $\mu$ l	1.0 ml	1.0 ml	1.0 ml
Distilled deionized water	15 ml	48.5 ml	33.5 ml	73.5 - (A)*
TEMED	25 $\mu$ l	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
10% APS	<u>125 <math>\mu</math>l</u>	<u>500 <math>\mu</math>l</u>	<u>500 <math>\mu</math>l</u>	<u>500 <math>\mu</math>l</u>
Total volume	25 ml	100 ml	100 ml	100 ml

Degas before polymerization

- \* The letter A designates the volume of 30% Acrylamide/Bis Solution required to produce the specified percent gel (X%).

## 4.2 Sequencing Gel<sup>2</sup>, 19:1 Ratio

	<b>4%</b>	<b>6%</b>	<b>X%</b>
30% Acrylamide/Bis 19:1	20 ml	30 ml	5(X%) = ml
10x TBE	15 ml	15 ml	15 ml
Urea	63 g	63 g	63 g
TEMED	150 $\mu$ l	150 $\mu$ l	150 $\mu$ l
25% APS	150 $\mu$ l	150 $\mu$ l	150 $\mu$ l

Adjust the volume to 150 ml with distilled deionized water.

Degas before polymerization.

## Section 5 References

1. Laemmli, U. K., *Nature*, **227**, 680-685 (1970).
2. Sequi-Gen<sup>®</sup> Nucleic Acid Sequencing Cell Instruction Manual, Bio-Rad Laboratories.

## Section 6 Product Information

<b>Catalog Number</b>	<b>Product Description</b>	<b>Quantity</b>
161-0154	<b>30% Acrylamide/Bis Solution, 19:1 ratio</b>	500 ml
161-0155		2 x 500 ml
161-0156	<b>30% Acrylamide/Bis Solution, 29:1 ratio</b>	500 ml
161-0157		2 x 500 ml
161-0158	<b>30% Acrylamide/Bis Solution, 37.5:1 ratio</b>	500 ml
161-0159		2 x 500 ml
161-0144	<b>40% Acrylamide/Bis Solution, 19:1 ratio</b>	500 ml
161-0145		2 x 500 ml
161-0146	<b>40% Acrylamide/Bis Solution, 29:1 ratio</b>	500 ml
161-0147		2 x 500 ml
161-0148	<b>40% Acrylamide/Bis Solution, 37.5:1 ratio</b>	500 ml
161-0149		2 x 500 ml
161-0140	<b>40% Acrylamide Solution</b>	500 ml
161-0141		2 x 500 ml
161-0142	<b>2% Bis Solution</b>	500 ml
161-0143		2 x 500 ml