Rhinohide™ Polyacrylamide Gel Strengthener

R33410 Rhinohide™ Polyacrylamide Gel Strengthener Kit
R33400 Rhinohide™ Polyacrylamide gel strengthener concentrate
A33405 acrylamide/bis-acrylamide mixture (37.5:1 ratio)

Quick Facts

Storage upon receipt:

- 2–25ºC

Introduction

Molecular Probes' Rhinohide™ polyacrylamide gel strengthener improves upon classic polyacrylamide gel technology by making gels much stronger, providing easier handling and resistance to tearing (Figure 1) without adverse side effects. Rhinohide™ polyacrylamide gel strengthener is highly recommended for low-percentage gels, large-format gels, and gels subject to multiple staining and handling steps.

SDS-polyacrylamide gels supplemented with Rhinohide™ polyacrylamide gel strengthener exhibit resolution capabilities comparable to traditional SDS-polyacrylamide gels, giving clear, focused bands. Rhinohide™ polyacrylamide gel strengthener reinforces a gel without the undesirable side effects common with other gel strengtheners.[1] For example, film-backed gels and polyester fabric–reinforced gels interfere with blotting techniques and can negatively affect protein staining. Alternatively, strengthening gels by the addition of pre-formed polymers causes turbidity and can produce serious spot-morphology artifacts, such as the distortion of high molecular weight bands or doubling of protein spots in the molecular weight dimension of 2-D gels.[2]

Rhinohide™ polyacrylamide gel strengthener produces gels with excellent transparency, providing exceptional image viewing and scanning of fluorescently stained gels, with minimal background staining.[3] Compatible with silver and Coomassie® staining, it is also the perfect companion to our Multiplexed Proteomics® platform, which includes SYPRO® Ruby, Pro-Q® Emerald, and Pro-Q® Diamond fluorescent gel stains for the detection of total protein, glycoproteins, and phosphoproteins, respectively. Our Multiplexed Proteomics® technologies make advanced proteome analysis attainable, simple, and affordable.

The Rhinohide™ gel strengthener is available in the Rhinohide™ Polyacrylamide Gel Strengthener Kit (R33410), which also includes premeasured acrylamide/bis-acrylamide (37.5:1) sufficient to make 1 L of a 30% acrylamide stock solution. The strengthener is also available in a concentrated form as the Rhinohide™ polyacrylamide gel strengthener concentrate (R33400), which can be added to existing stock solutions of acrylamide/bis-acrylamide (37.5:1). Molecular Probes also offers the acrylamide/bis-acrylamide mixture (A33405) separately for making these stock solutions.

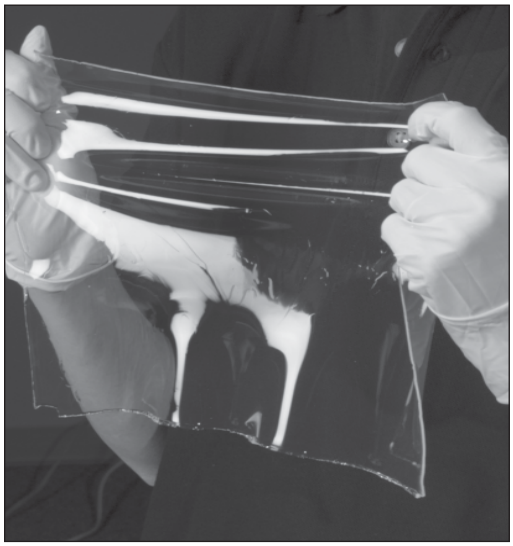

Figure 1. Gels made with Rhinohide™ polyacrylamide gel strengthener are extremely strong and durable.

Contents and Storage

Rhinohide™ Polyacrylamide Gel Strengthener Kit (R33410)

- **Rhinohide™ polyacrylamide gel strengthener** (Component A), 724 mL
- **Acrylamide/bis-acrylamide mixture (37.5:1 ratio)** (Component B), 300 g

This kit provides sufficient materials for making 1 L of a 30% acrylamide/bis-acrylamide stock solution containing the Rhinohide™ gel strengthener; this stock solution can be used to cast gels from 5% to 20% (see protocols, below). Upon receipt, the kit should be stored at 2–25ºC and should be stable for at least 6 months (longer if stored at 2–6ºC). WARNING: Acrylamide is toxic and should be handled with appropriate precautions.

Table 1. Gel preparation using Rhinohide™ gel strengthener premixed in a 30% acrylamide/bis-acrylamide (37.5:1) stock solution.

Ingredient *	Resolving Gel							Stacking Gel
	5%	7.5%	10%	12.5%	15%	17.5%	20%	4%
Rhinohide™ strengthener in 30% acrylamide stock solution †	16.7	25.0	33.3	41.7	50.0	58.3	66.7	1.33
Buffer ‡	25.0	25.0	25.0	25.0	25.0	25.0	25.0	2.50
dH_2O	57.0	48.7	40.4	32.0	23.7	15.4	7.00	6.01
10% SDS §	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.100
10% APS **	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.050
TEMED **	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.010
Total volume	100.0	100.0	100.0	100.0	100.0	100.0	100.0	10.0

***** Volumes in mL for making the indicated polyacrylamide percentage; ingredient volumes may be scaled up or down for different total volumes. **†** The 30% acrylamide/bis-acrylamide must be in a 37.5:1 ratio. **‡** The buffer ingredient for standard SDS-polyacrylamide resolving gels is 1.5 M Tris-HCl, pH 8.8; the buffer ingredient for stacking gels is 0.5 M Tris-HCl, pH 6.8. **§** De-gas the first three ingredients before adding the 10% SDS. ****** Add 10% APS and TEMED only immediately before use.

Rhinohide™ Polyacrylamide Gel Strengthener Concentrate (R33400)

The Rhinohide™ polyacrylamide gel strengthener concentrate is supplied in a unit size of 200 mL. As supplied, the concentrate provides sufficient additive to make 1 L of a 30% acrylamide/bis-acrylamide (37.5:1) stock solution containing the Rhinohide™ gel strengthener (see protocols, below). In this case, the user must supply the acrylamide/bis-acrylamide mixture (37.5:1), but the end result is a solution identical to the solution made with our Rhinohide™ Gel Strengthener Kit. Note that the Rhinohide™ polyacrylamide gel strengthener concentrate is 3.62-fold more concentrated than Component A of the Rhinohide™ Polyacrylamide Gel Strengthener Kit. The Rhinohide™ polyacrylamide gel strengthener concentrate should be stable for at least 6 months when stored at 2–25˚C (longer if stored at 2–6ºC).

Acrylamide/Bis-Acrylamide Mixture (37.5:1 ratio) (A33405)

The acrylamide/bis-acrylamide mixture (37.5:1 ratio) is supplied in a unit size of 300 g — 292.2 g of acrylamide and 7.8 g of bis-acrylamide (*N*,*N*′-methylene-bis-acrylamide). Upon receipt, the mixture should be stored at 2–25ºC and should be stable for at least one year. Note that the acrylamide/bis-acrylamide mixture can be used with Rhinohide™ polyacrylamide gel strengthener concentrate to make 1 L of a 30% acrylamide/bis-acrylamide stock solution containing the Rhinohide™ gel strengthener (see protocols, below). Alternatively, the mixture can be used to make a conventional 30% stock solution in water (see protocols, below). Store the solutions at 2–6ºC, where they should be stable for at least 6 months. WARNING: Acrylamide is toxic and should be handled with appropriate precautions.

Materials Required but Not Provided

- 1.5 M Tris-HCl, pH 8.8
- 0.5 M Tris-HCl, pH 6.8
- 10% Sodium dodecylsulfate (SDS)
- 10% Ammonium persulfate (APS)
- TEMED

Experimental Protocols

Rhinohide™ Polyacrylamide Gel Strengthener Kit (R33410)

1.1 Prepare a 1 L solution of 30% acrylamide/bis-acrylamide (37.5:1) containing the Rhinohide™ gel strengthener by pouring the entire contents of the Rhinohide™ polyacrylamide gel

Table 2. Gel preparation using Rhinohide™ gel strengthener concentrate and a 30% acrylamide/bis-acrylamide (37.5:1) stock solution.

Ingredient *	Resolving Gel							Stacking Gel
	5%	7.5%	10%	12.5%	15%	17.5%	20%	4%
30% acrylamide stock solution †	16.7	25.0	33.3	41.7	50.0	58.3	NA	1.33
Rhinohide™ concentrate	3.33	5.00	6.67	8.33	10.0	11.7	NA	0.267
Buffer ‡	25.0	25.0	25.0	25.0	25.0	25.0	NA	2.50
dH_2O	53.7	43.7	33.7	23.7	13.7	3.70	NA	5.74
10% SDS §	1.00	1.00	1.00	1.00	1.00	1.00	NA	0.100
10% APS **	0.250	0.250	0.250	0.250	0.250	0.250	NA	0.050
TEMED **	0.050	0.050	0.050	0.050	0.050	0.050	NA	0.010
Total volume	100.0	100.0	100.0	100.0	100.0	100.0	NA	10.00

***** Volumes in mL for making the indicated polyacrylamide percentage; ingredient volumes may be scaled up or down for different total volumes. **†** The 30% acrylamide/bis-acrylamide must be in a 37.5:1 ratio. **‡** The buffer ingredient for standard SDS-polyacrylamide resolving gels is 1.5 M Tris-HCl, pH 8.8; the buffer ingredient for stacking gels is 0.5 M Tris-HCl, pH 6.8. **§** De-gas the first three ingredients before adding the 10% SDS. ****** Add 10% APS and TEMED only immediately before use. NA, not applicable.

Table 3. Gel preparation using Rhinohide™ gel strengthener concentrate and a 40% acrylamide/bis-acrylamide (37.5:1) stock solution.

Ingredient *	Resolving Gel							Stacking Gel
	5%	7.5%	10%	12.5%	15%	17.5%	20%	4%
40% acrylamide stock solution †	12.5	18.8	25.0	31.3	37.5	43.8	50.0	1.00
Rhinohide™ concentrate	3.33	5.00	6.67	8.33	10.0	11.7	13.3	0.267
Buffer ‡	25.0	25.0	25.0	25.0	25.0	25.0	25.0	2.50
dH_2O	57.9	49.9	42.0	34.1	26.2	18.2	10.4	6.07
10% SDS §	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.100
10% APS **	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.050
TEMED **	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.010
Total volume	100.0	100.0	100.0	100.0	100.0	100.0	100.0	10.00

***** Volumes in mL for making the indicated polyacrylamide percentage; ingredient volumes may be scaled up or down for different total volumes. **†** The 40% acrylamide/bis-acrylamide must be in a 37.5:1 ratio. **‡** The buffer ingredient for standard SDS-polyacrylamide resolving gels is 1.5 M Tris-HCl, pH 8.8; the buffer ingredient for stacking gels is 0.5 M Tris-HCl, pH 6.8. **§** De-gas the first four ingredients before adding the 10% SDS. ****** Add 10% APS and TEMED only immediately before use.

strengthener (Component A) into the bottle containing the premeasured acrylamide/bis-acrylamide mixture (Component B). Stir the solution (using a magnetic stirrer and stir bar) for 2–4 hours. Inspect the solution to ensure the acrylamide and bis-acrylamide have dissolved.

This stock solution can be stored at 2–6˚C for at least one year.

1.2 Use the 30% acrylamide/bis-acrylamide stock solution containing the Rhinohide™ gel strengthener (prepared in step 1.1) to prepare polyacrylamide gels, as described in Table 1.

Rhinohide™ Gel Strengthener Concentrate (R33400)

There are two methods for incorporating the Rhinohide™ gel strengthener concentrate into polyacrylamide gels. The first is to add a small amount of the Rhinohide™ polyacrylamide gel strengthener concentrate directly to the acrylamide solutions as the polymerization mixture is prepared. The Rhinohide™ polyacrylamide gel strengthener concentrate can be used with a conventional 30% or 40% acrylamide/bis-acrylamide (37.5:1) solution. Refer to Table 2 for recipes using a 30% acrylamide/bis-acrylamide (37.5:1) stock solution and to Table 3 for recipes using a 40% acrylamide/bis-acrylamide (37.5:1) stock solution.

The second method for incorporating the Rhinohide™ polyacrylamide gel strengthener concentrate into the gel mixture is to prepare a 30% acrylamide/bis-acrylamide (37.5:1) stock solution containing the Rhinohide™ gel strengthener. (The acrylamide/bis-acrylamide is not supplied with the Rhinohide™ polyacrylamide gel strengthener concentrate.)

2.1. Prepare a 30% acrylamide/bis-acrylamide solution containing Rhinohide™ gel strengthener by adding 200 mL of the Rhinohide™ gel strengthener concentrate to 300 g of premeasured acrylamide/bis-acrylamide (37.5:1 ratio) in a 1 L container. To this mixture add 524 mL of deionized water (dH_2O). Stir the solution (using a magnetic stirrer and stir bar) for 2–4 hours. Inspect the solution to ensure the acrylamide and bis-acrylamide have dissolved. This stock solution can be stored at 2–6˚C for at least six months.

2.2. Use the 30% acrylamide/bis-acrylamide stock solution containing the Rhinohide™ gel strengthener to prepare polyacrylamide gels, as described in Table 1.

Acrylamide/Bis-Acrylamide Mixture (37.5:1 ratio) (A33405)

There are two methods for preparing 1 L of a 30% acrylamide/bis-acrylamide stock solution from the premeasured mixture. In the first method, the 30% stock solution is prepared in water alone.

3.1 Add 724 mL of dH_2O to the bottle of premeasured acrylamide/bis-acrylamide. Stir the solution (using a magnetic stirrer and stir bar) for 2–4 hours. Inspect the solution to ensure the acrylamide and bis-acrylamide have dissolved. Store the solution at 2–6°C, where it should be stable for 6 months.

3.2 Use this stock solution for making polyacrylamide gels that include the Rhinohide™ gel strengthener by following the protocol described in Table 2.

In the second method, the 30% stock solution is prepared with the inclusion of the Rhinohide™ polyacrylamide gel strengthener concentrate.

4.1 Add 200 mL of the Rhinohide™ polyacrylamide gel strengthener concentrate (R33400) and 524 mL of dH_2O to the bottle of premeasured acrylamide/bis-acrylamide. Stir the solution (using a magnetic stirrer and stir bar) for 2–4 hours. Inspect the solution to ensure the acrylamide and bis-acrylamide have dissolved. Store the solution at 2–6°C, where it should be stable for 6 months.

4.2 Use this stock solution for making polyacrylamide gels that include the Rhinohide™ gel strengthener by following the protocol described in Table 1.

WARNING: Acrylamide is toxic and should be handled with appropriate precautions.

References

1. Anal Biochem 148, 384 (1985); **2.** Electrophoresis 21, 486 (2000); **3.** Proteomics 3, 1196 (2003).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
R33400	Rhinohide™ polyacrylamide gel strengthener concentrate *sufficient additive for 1 L of 30% acrylamide/bis-acrylamide (37.5:1)*	200 mL
R33410	Rhinohide™ Polyacrylamide Gel Strengthener Kit *makes 1 L of Rhinohide™ 30% acrylamide/bis-acrylamide (37.5:1)*	1 kit
A33405	acrylamide/bis-acrylamide mixture (37.5:1 ratio) *electrophoresis grade*	300 g

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

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