# TGX<sup>™</sup> and TGX Stain-Free<sup>™</sup> FastCast<sup>™</sup> Acrylamide Kit and Starter Kit Instruction Manual

TGX and TGX Stain-Free FastCast acrylamide kits are solutions for hand casting gels for SDS-PAGE or PAGE use. The unique formulation enables:

- Ability to cast resolving and stacking gels at the same time and start running your gels in as little as 30 min
- Ability to conduct electrophoretic runs as fast as 20 min (at 300 V) without damage to the handcast gels
- Efficient protein transfers onto blot in as little as 3 min (using the Trans-Blot<sup>®</sup> Turbo<sup>™</sup> blotter)
- 1 year of shelf life at room temperature (22–28°C) for acrylamide solutions and up to 4 weeks at 4°C for cast gels
- Stain-free visualization of proteins in your gel or blot in less than 5 min with TGX Stain-Free kits
- Ability to monitor your electrophoresis and transfer steps without additional staining using TGX Stain-Free kits

Table 1. Approximate number of gels per kit.

	Mini Gels (~9 x 7 cm)				Midi Gels (~13 x 9 cm)
	1.0 mm Mini-PROTEAN® Cassettes	0.75 mm Glass Plates	1.0 mm Glass Plates	1.5 mm Glass Plates	1.0 mm Criterion™ Cassettes
Starter Kit	11 gels	19 gels	13 gels	9 gels	6 gels
Kit	55 gels	95 gels	65 gels	45 gels	30 gels

When casting different numbers of gels, prepare the solutions according to Table 2 and follow the instructions.

The starter kits enable mixing the stacker A and B and resolver A and B solutions without calculation and casting at least 10 Mini-PROTEAN gels at one time.

Note: After adding APS and TEMED to the resolver solution mixture, casting should be started immediately and combs inserted within 8–9 min. To ensure sufficient time to insert combs, do not cast more than 10 gels at one time.

#### Table 2. Casting preparation volumes.

	Mini-PI Empty C	mm ROTEAN Cassettes gels)	Bio- Glass	mm Rad Plates gels)	Bio Glass	mm -Rad Plates gels)	1.5 Bio- Glass (n = g	Rad Plates	1.0 r Crite Empty Ca (n = g	erion assettes
	Stacker	Resolver	Stacker	Resolver	Stacker	Resolver	Stacker	Resolver	Stacker	Resolver
Resolver A	-	3.5 ml x n	-	2 ml x n	-	3 ml x n	-	4 ml x n	-	6 ml x n
Resolver B	-	3.5 ml x n	-	2 ml x n	-	3 ml x n	-	4 ml x n	-	6 ml x n
Stacker A	1 ml x n	-	1 ml x n	-	1 ml x n	-	1.5 ml x n	-	1.5 ml x n	-
Stacker B	1 ml x n	-	1 ml x n	-	1 ml x n	-	1.5 ml x n	-	1.5 ml x n	-
Total Volume	2 ml x n	<b>7 ml</b> x n	2 ml x n	4 ml x n	2 ml x n	6 ml x n	<b>3 ml</b> x n	8 ml x n	<b>3 ml</b> x n	<b>12 ml</b> x n
TEMED	2 µl x n	3.5 µl x n	2 µl x n	2 µl x n	2 µl x n	3 µl x n	3 µl x n	4 µl x n	3 µl x n	6 µl x n
10% APS	10 µl x n	35 µl x n	10 µl x n	20 µl x n	10 µll x n	30 µl x n	15 µl x n	40 µl x n	15 µl x n	60 µl x n

#### Instructions

- 1. Prepare resolving gel acrylamide solution by combining equal volumes of resolver A and B solutions (see Table 2). Note: To use all the solutions from the starter kits, pour 40 ml resolver B solution directly into the larger amber bottle containing 40 ml resolver A solution.
- If using the Mini-PROTEAN plastic cassettes, they should be held vertically for gel casting. Use the single-row AnyGel<sup>™</sup> casting stand (catalog #165-4131) to stabilize the cassette during casting. For Bio-Rad handcast glass plates, use the Mini-PROTEAN Tetra cell casting stand (catalog #165-8051 or #165-8052) to stabilize the plates during casting.
- 3. Add the required volume of TEMED and freshly made 10% APS to the combined resolver solution and mix well. Use an appropriate pipet to steadily dispense the solution into the cassette. Do not let bubbles form or solution mix with air. Fill the cassette to 0.5–1 cm below the bottom of the teeth on the comb. Immediately prepare and pour the stacking solution as directed in the next steps.

**Note**: Insert a comb and mark the cassette to ensure the resolving solution is poured to the desired distance below the comb. **Note**: For the combined resolving gel solutions from the starter kits, add  $40 \ \mu l$  of TEMED and  $400 \ \mu l$  of freshly made 10% APS.

- 4. Prepare stacking gel acrylamide solution by combining equal volumes of stacker A and B solutions (see Table 2). Note: To use all the solutions from the starter kits, pour the 15 ml stacker B solution directly into the larger amber bottle containing 15 ml stacker A solution.
- 5. Add required volume of TEMED and freshly made 10% APS to the combined stacker solution and mix well. Pipet solution down the middle of the cassette, filling to the top of the short plates. Apply slowly and steadily to prevent mixing with the resolving solution. Align and insert the comb in the cassette carefully to prevent air from being trapped under the comb teeth. Allow the gel to polymerize for 30–45 min before electrophoresis.

Note: For the combined stacking gel solutions from the starter kit, add 30 µl of TEMED and 150 µl of freshly made 10% APS.



- The cast gels can be covered with a paper towel wetted with deionized water and stored in a zipper sealed plastic bag at 4°C for up to 4 weeks.
- 7. Follow the guideline below for gel electrophoresis, stain-free imaging, and western blot transfer. Refer to the Mini-PROTEAN Precast Gels Instruction Manual and Application Guide (#1658100) for details on running, handling, and other applications of TGX and TGX Stain-Free gels.

Use Laemmli sample buffer (with or without reducing agents such as BME) to prepare samples for loading and 1x Tris/glycine/SDS running buffer to run the TGX gels.

### Table 3. Running conditions for handcast TGX or TGX Stain-Free SDS-PAGE gels in the Mini-PROTEAN Tetra cell.

	100 V Low voltage	200 V Standard	300 V Rapid
Run time	85–95 min	30–40 min	20–25 min
Lower chamber buffer volume (for 2 gels)	550 ml	550 ml	800 ml
Lower chamber buffer volume (for 4 gels)	800 ml	800 ml	800 ml

Note:

• When running only 1-2 gels in the Mini-PROTEAN Tetra cell, do not leave the companion module in the tank

 Do not run different gel types (chemistry) or percentages in the same tank at the same time

### Table 4. Stain-Free gel activation using a Bio-Rad Stain-Free enabled imager.

	Gels used in	Good*	Best*
	Blotting	Sensitivity	Sensitivity
Activation time	1 min	2.5 min	5 min

\* For gels not used in blotting.

Note:

- Must activate stain-free compound in gel for stain-free blot imaging
- Stain-free activation protocols can be accessed in Bio-Rad's Image Lab<sup>™</sup> software

## Table 5. Transfer conditions for blotting in the Trans-Blot Turbo system.

Protocol Name	MW, kD	Time, min	2 Mini Gels or 1 Midi Gel	1 Mini Gel	
Standard SD	Standard SD Any 30		Up to 1.0 A; 25 V, constant		
1.5 mm gel	Any	10			
High MW	>150	10	2.5 A constant	1.3 A constant	
Low MW	<30	5	up to 25 V	up to 25 V	
Mixed MW*	5–150	7			
1 Mini TGX*	5–150	3	N/A	2.5 A constant up to 25 V	

\* Also accessed via Turbo navigation button.

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#### **Ordering Information**

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Catalog #	Description
161-0170	TGX <sup>™</sup> FastCast <sup>™</sup> Acrylamide Starter Kit, 7.5%
161-0171	TGX FastCast Acrylamide Kit, 7.5%
161-0172	TGX FastCast Acrylamide Starter Kit, 10%
161-0173	TGX FastCast Acrylamide Kit, 10%
161-0174	TGX FastCast Acrylamide Starter Kit, 12%
161-0175	TGX FastCast Acrylamide Kit, 12%
161-0180	TGX Stain-Free <sup>™</sup> FastCast <sup>™</sup> Acrylamide Starter Kit, 7.5%
161-0181	TGX Stain-Free FastCast Acrylamide Kit, 7.5%
161-0182	TGX Stain-Free FastCast Acrylamide Starter Kit, 10%
161-0183	TGX Stain-Free FastCast Acrylamide Kit, 10%
161-0184	TGX Stain-Free FastCast Acrylamide Starter Kit, 12%
161-0185	TGX Stain-Free FastCast Acrylamide Kit, 12%
161-0700	Ammonium Persulfate (APS), 10 g
161-0800	TEMED, 5 ml
	is are 4-gel with 2 casting stands, 4 casting frames, s, 5 short plates, and 5 spacer plates.
165-8000	Mini-PROTEAN <sup>®</sup> Tetra Cell and System, 0.75 mm gel thickness
165-8001	Mini-PROTEAN Tetra Cell and System, 1.0 mm gel thickness
165-8006	Mini-PROTEAN Tetra Cell and System, 1.5 mm gel thickness
Standards Val	ue Pack (5 Packs)
161-0393	Precision Plus Protein <sup>™</sup> All Blue Standards; 10–250 kD
161-0394	Precision Plus Protein Dual Color Standards; 10–250 kD
161-0395	Precision Plus Protein <sup>™</sup> Kaleidoscope <sup>™</sup> Standards; 10–250 kD
161-0397	Precision Plus Protein Dual Xtra Standards; 2–250 kD
161-0396	Precision Plus Protein Unstained Standards; 10–250 kD
161-0737	2x Laemmli Sample Buffer, 30 ml
161-0747	4x Laemmli Sample Buffer, 10 ml
161-0772	10x Tris/Glycine/SDS Buffer, 5 L
Mini-PROTFA	N Empty Cassettes and Combs, pkg of 50
456-0001	Mini-PROTEAN Empty Cassettes, IPG well marks
456-0003	Mini-PROTEAN Empty Cassettes, 10-well marks
456-0005	Mini-PROTEAN Empty Cassettes, 12-well marks
456-0005	Mini-PROTEAN Empty Cassettes, 12-well marks
456-0001	Mini-PROTEAN Combs, IPG well combs
456-0013	Mini-PROTEAN Combs, 10-well combs
456-0015	Mini-PROTEAN Combs, 12-well combs
456-0016	Mini-PROTEAN Combs, 12-well combs
	ty Cassettes, pkg of 10 cassettes with combs
345-9901	Criterion Empty Cassettes, 12+2 well
345-9902	Criterion Empty Cassettes, 18-well
345-9903	Criterion Empty Cassettes, 10 well
345-9904	Criterion Empty Cassettes, Prep+2
345-9906	Criterion Empty Cassettes, IPG+1

\* Please see the accessories tab on www.bio-rad.com/tetra for individual handcast products.



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