User Guide

TurboMix™ Bis-Tris Gel Casting Kit

TMKIT-10
TMKIT-60

Introduction

The TurboMix™ Bis-Tris Gel Casting Kit is comprised of a Resolving Solution and a Stacking Solution. The solutions have been optimized to simplify gel preparation steps and minimize reagent waste. The TurboMix™ solutions can be used in traditional casting methods or the Quick Cast procedure.

The Quick Cast procedure polymerizes in one step by pouring the stacking gel immediately after the resolving gel. TurboMix™ Resolving Solution is provided at 20% acrylamide and formulated for dilution with deionized water, which allows the flexibility to cast different resolving gel percentages. The TurboMix™ Stacking Solution is provided at 4% acrylamide and requires only the addition of Ammonium persulfate (APS) and N,N',N'-tetramethylethane-1,2-diamine (TEMED). Review the entire user guide before first use.

Kit Components

- TurboMix™ Resolving Solution (20% acrylamide)
- TurboMix™ Stacking Solution (4% acrylamide)

Additional materials required (not provided)

- TEMED
- 10% APS
- Gel casting stand and plates
- 5 mL sterile serological pipettes
- MOPS-SDS running buffer or MES-SDS running buffer
- 4X LDS Sample Buffer

Storage

Store bottles at 2 °C - 8 °C. Protect Resolving Solution and Stacking Solution from light. Storing solutions in kit box is recommended. See package label for expiration date.

Important:

- Bis-Tris gels are not compatible with Tris-Glycine-SDS running buffer. Only use MOPS or MES running buffers with this kit.
- Clean casting equipment of any acrylamide residue to ensure proper polymerization and inspect glass plates for nicks that could result in leakage.
- The volume of Resolving Solution and Stacking Solution required will depend on the gel size and thickness. Tables 1-3 provide examples of reagent preparation with the TurboMix™ Gel Casting kit. Users may need to empirically determine the required volumes.

Volumes Required To Cast One Mini Gel (7.4 cm x 8.2 cm)

Table 1
1 mm thick mini gel

	Resolving Gel				Stacking Gel
Gel percentage	**8%**	**10%**	**12%**	**15%**	**4%**
TurboMix™ Resolving solution	2.4 mL	3 mL	3.6 mL	4.5 mL	N/A
TurboMix™ Stacking solution	N/A	N/A	N/A	N/A	2 mL
D.I. water	3.6 mL	3 mL	2.4 mL	1.5 mL	N/A
10% APS	30 µL	30 µL	30 µL	30 µL	20 µL
TEMED	3 µL	3 µL	3 µL	3 µL	2 µL

Table 2
0.75 mm thick mini gel

	Resolving Gel				Stacking Gel
Gel percentage	**8%**	**10%**	**12%**	**15%**	**4%**
TurboMix™ Resolving solution	1.8 mL	2.25 mL	2.7 mL	3.38 mL	N/A
TurboMix™ Stacking solution	N/A	N/A	N/A	N/A	1.5 mL
D.I. water	2.7 mL	2.25 mL	1.8 mL	1.12	N/A
10% APS	22.5 µL	22.5 µL	22.5 µL	22.5 µL	15 µL
TEMED	2.25 µL	2.25 µL	2.25 µL	2.25 µL	1.5 µL

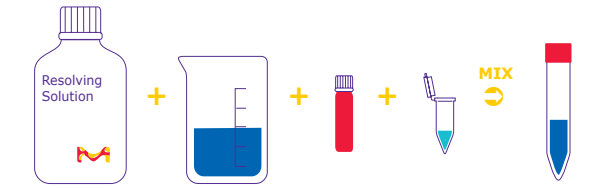
Table 3
1.5 mm thick mini gel

	Resolving Gel				Stacking Gel
Gel percentage	**8%**	**10%**	**12%**	**15%**	**4%**
TurboMix™ Resolving solution	3.6 mL	4.5 mL	5.4 mL	6.75 mL	N/A
TurboMix™ Stacking solution	N/A	N/A	N/A	N/A	3 mL
D.I. water	5.4 mL	4.5 mL	3.6 mL	2.25 mL	N/A
10% APS	45 µL	45 µL	45 µL	45 µL	30 µL
TEMED	4.5 µL	4.5 µL	4.5 µL	4.5 µL	3 µL

Quick Cast Instructions

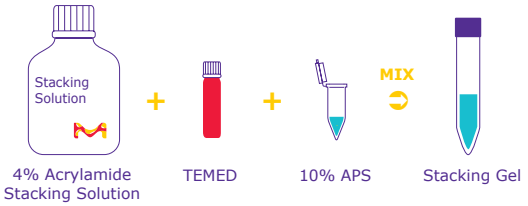
1. Clean and set up casting equipment.
2. Mark desired height of resolving gel, usually 0.5-1 cm below the bottom of the comb teeth.
3. Prepare 10% APS from powder. Note: 10% APS can be prepared fresh with every use or aliquoted and stored at -20 °C. Avoid repeated freeze-thaw cycles.
4. Use Table 1, 2, or 3 depending on the cassette thickness to calculate the volume of reagents required. Multiply volumes by the desired number of gels to cast multiple gels at once. You may cast up to 4 gels at once using the Quick Cast method.
5. Prepare the desired resolving gel percentage by pipetting TurboMix™ Resolving Solution, D.I. water, 10% APS and TEMED into a clean conical tube. To avoid contamination, use a sterile serological pipette to draw TurboMix™ solutions from stock bottles.

   NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.

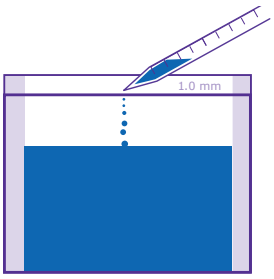


6. Gently mix reagents by inverting conical tube, avoiding introduction of air bubbles into the gel mixture.
7. Prepare the stacking gel by pipetting TurboMix™ Stacking Solution into a clean, separate conical tube and adding the required amount of TEMED and 10% APS.

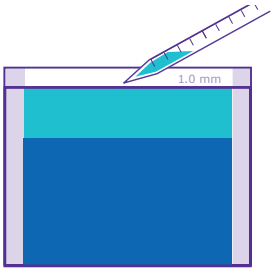
   NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.



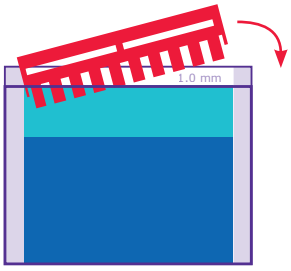
8. Gently mix reagents by inverting conical tube, avoiding introduction of air bubbles into the gel mixture.
9. Using a serological pipette, fill each cassette to marked height with resolving gel.



10. Position serological pipette at the middle of the cassette and gently add the stacking gel, filling to the top of the short plate. A dip may occur where pipetting takes place but will level out.



11. Quickly and carefully insert the comb. Inserting at an angle may help to avoid trapping air bubbles below the teeth.



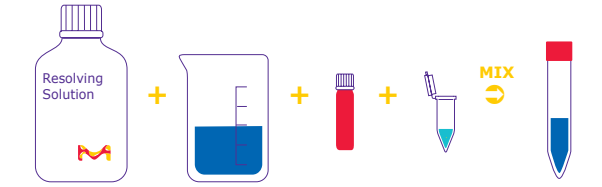
12. Allow gels to polymerize for 1 hour. Left over casting solution can be used to monitor polymerization.

Gels can be used immediately or wrapped in D.I. water-soaked paper towels and stored in an air-tight container at 4 °C for up to 4 weeks.

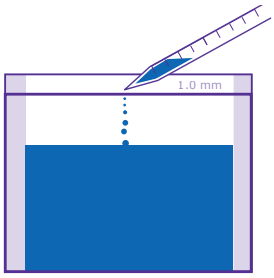
Traditional Cast Instructions

1. Clean and set up casting equipment.
2. Mark desired height of resolving gel, usually 0.5-1 cm below the bottom of the comb teeth.
3. Prepare 10% APS from powder. Note: 10% APS can be prepared fresh with every use or aliquoted and stored at -20 °C. Avoid repeated freeze-thaw cycles.
4. Use Table 1, 2, or 3 depending on the cassette thickness to calculate the volume of reagents required. Multiply volumes by the desired number of gels to cast multiple gels at once.
5. Prepare the desired resolving gel percentage by pipetting TurboMix™ Resolving Solution, D.I. water, APS and TEMED into a clean conical tube. To avoid contamination, use a sterile serological pipette to draw TurboMix™ solutions from stock bottles.

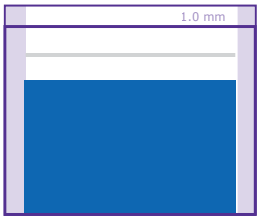
   NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.



6. Gently mix reagents by inverting conical tube, avoiding introduction of air bubbles into gel mixture.

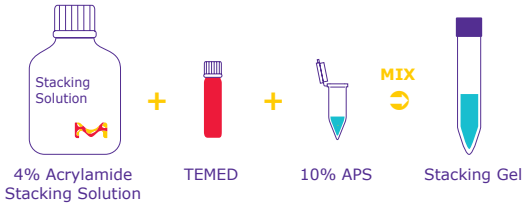


7. Using a serological pipette, fill each cassette to marked height with resolving gel.
8. Carefully overlay with isopropanol. Allow resolving gel to polymerize, approximately 30 minutes. A sharp line will appear below the isopropanol as acrylamide polymerizes. Alternatively, left over casting solution can be used to monitor polymerization.

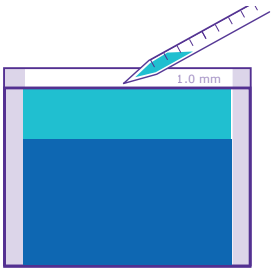


9. Remove the isopropanol and rinse with deionized water. Blot with filter paper to remove residual water.
10. To prepare the stacking gel, pipette the required amount of TurboMix™ Stacking Solution, TEMED and 10% APS into a clean conical.

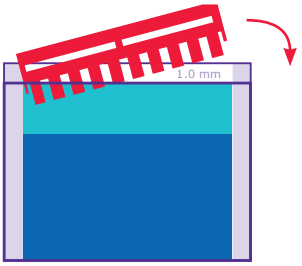
    NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.



11. Gently mix reagents by inverting conical tube, avoiding introduction of air bubbles. Pipette stacking gel into cassettes and fill to the top of the short plate.



12. Quickly and carefully insert the comb. Inserting at an angle may help to avoid trapping air bubbles below the teeth.



13. Allow gels to polymerize for 1 hour.

Gels can be used immediately or wrapped in DI water-soaked paper towels and stored in an air-tight container at 4 °C for up to 4 weeks.

Sample Preparation and Electrophoresis

1. Samples should be prepared just prior to electrophoresis, according to Table 4.

   Note: Do not store reduced samples for >2 hours as they may reoxidize.

2. Heat samples for 10 minutes at 70 °C then briefly centrifuge. Do not boil samples.
3. Install gel into the electrophoresis tank and add MOPS or MES running buffer.
4. Remove the comb from the gel and gently rinse wells with running buffer.
5. Load samples. Close the tank with lid and connect leads to an appropriate power supply.
6. The gel can be run at 200V.

Table 4
Preparation of Electrophoresis Samples Reagent Reduced Sample

Reagent	Reduced Sample (µL)	Non-reduced sample (µL)
Protein sample	X	X
4X LDS Sample Buffer	2.5	2.5
1M-DTT	1	N/A
Deionized water	6.5-X	7.5-X
Total Volume	10	10

Buffer Formulation

Note: use either MOPS or MES running buffer. Do not use Tris-Glycine running buffer with TurboMix gels.

4X LDS Sample Buffer
(Cat. No. MPSB-10ML or MPSB-250ML)

Reagent	Amount
Tris-HCl	0.666 g
Tris-Base	0.682 g
Lithium Dodecyl Sulfate (LDS)	0.800 g
EDTA	0.006 g
Glycerol	4 g
Coomassie Brilliant Blue G250 (1% solution)	0.75 mL
Phenol Red (1% solution)	0.25 mL
Deionized water	to 10 mL

1X mPAGE MOPS SDS Running Buffer
(Cat. No. MPM0PS)

Reagent	Amount
MOPS	10.46 g
Tris-Base	6.06 g
SDS	1.00 g
EDTA	0.30 g
Deionized water	to 1 L

1X mPAGE MES SDS Running Buffer
(Cat. No. MPMES)

Reagent	Amount
MES	9.76 g
Tris-base	6.06 g
SDS	1.00 g
EDTA	0.30 g
Deionized water	to 1 L

Product Ordering

Purchase online at SigmaAldrich.com/products.

Description	Qty/Pk	Catalogue Number
TurboMix™ Bis-Tris Gel Casting Kit	~10 mini gels	TMKIT-10
TurboMix™ Bis-Tris Gel Casting Kit	~60 mini gels	TMKIT-60
TurboMix™ Resolving Solution	216 mL	TMRES-216ML
TurboMix™ Stacking Solution	120 mL	TMSTK-120ML
Buffers		
mPAGE™ 4X LDS Sample Buffer	10 mL	MPSB-10ML
mPAGE™ 4X LDS Sample Buffer	250 mL	MPSB-250ML
mPAGE™ MES SDS Running Buffer Powder (Each packet makes 1L)	5 packets	MPMES
mPAGE™ MOPS SDS Running Buffer Powder (Each packet makes 1L)	5 packets	MPM0PS
mPAGE™ Transfer Buffer Powder (Each packet makes 1L)	10 packets	MPTRB
Protein Markers		
mPAGE™ Color Protein Standard	500 µL	MPSTD4
mPAGE™ Unstained Protein Standard	500 µL	MPSTD3
mPAGE™ Western Protein Standard	250 µL	MPSTD2
Protein Gel Stains		
EZBlue™ Gel Staining Reagent	500 mL	G1041-500ML
ReadyBlue™ Protein Gel Stain	1 L	RSB-1L
EZFluor™ 1-step Fluorescent Protein Gel Stain	1 L	SCT145-1L
EZFluor™ UV 1-step Fluorescent Protein Gel Stain	1 L	SCT147-1L
ProteoSilver™ Silver Stain Kit	1 kit for 25 mini-gel	PROTSIL1-1KT

Description	Qty/Pk	Catalogue Number
Reagents		
Ammonium persulfate (APS) for molecular biology, for electrophoresis, ≥98%	25 g	A3678-25G
N,N,N′,N′-Tetramethylethylenediamine (TEMED) ReagentPlus®, 99%	5 mL	T22500-5ML
DL-Dithiothreitol solution, 1 M	10 mL	43816-10ML
2-Mercaptoethanol (BME)	25 mL	63689-25ML-F
Tris-HCl		T3253
Tris Base		T1503
Lithium dodecyl sulfate (LDS)		L9781
Sodium dodecyl sulfate (SDS)		L3771
Ethylenediaminetetraacetic acid (EDTA)		E5134
Glycerol		G2025
Phenol Red		114529
Coomassie Brilliant Blue G		B0770
Ethylenediaminetetraacetic acid (MOPS)		M1254
2-Morpholinoethanesulfonic acid monohydrate (MES)		M3671

Description	Qty/Pk	Catalogue Number
Transfer Membrane		
Immobilon®-E Membrane, PVDF, 0.45 µm, 8.5 cm x 10 m	1 roll	IEVH85R
Immobilon®-E PVDF Transfer Membranes, 7 cm x 8.4 cm sheet	50 sheets	IEVH07850
Immobilon®-P Membrane, PVDF, 0.45 µm, 8.5 cm x 10 m	1 roll	IPVH85R
Immobilon®-P PVDF Transfer Membranes, 7 cm x 8.4 cm sheet	50 sheets	IPVH07850
Immobilon®-FL Membrane, PVDF, 0.45 µm, 8.5 cm x 10 m	1 roll	IPFL85R
Immobilon®-PSQ Membrane, PVDF, 0.2 µm, 8.5 cm x 10 m	1 roll	ISEQ85R
Immobilon®-PSQ PVDF Transfer Membranes, 7 cm x 8.4 cm sheet	50 sheets	ISEQ07850
Electrophoresis Equipment		
mA400 Basic Power Supply, US plug	1 unit	MA400-US
mA400 Basic Power Supply, Euro plug	1 unit	MA400-EU
mA400 Basic Power Supply, UK plug	1 unit	MA400-UK
mA400 Basic Power Supply, Japan plug	1 unit	MA400-NI
mA400 Basic Power Supply, China plug	1 unit	MA400-ZH
mA700 Essential Power Supply, US plug	1 unit	MA700-US
mA700 Essential Power Supply, Euro plug	1 unit	MA700-EU
mA700 Essential Power Supply, UK plug	1 unit	MA700-UK
mA700 Essential Power Supply, Japan plug	1 unit	MA700-NI
mA700 Essential Power Supply, China plug	1 unit	MA700-ZH

Notice

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Standard Warranty

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