User Guide

mPAGE® TurboMix Bis-Tris Gel Casting Kit

TMKIT-10
TMKIT-60
TMRES-216ML
TMSTK-120ML

MERCK

Introduction

The mPAGE® TurboMix Bis-Tris Gel Casting Kit is comprised of a Resolving Solution and a Stacking Solution. These solutions have been optimized to simplify gel preparation and minimize reagent waste. The mPAGE® TurboMix Bis-Tris Gel Casting Kit can be used with either Quick Cast or Traditional Casting methods.

The Quick Cast procedure polymerizes in one step by pouring the stacking gel immediately after the resolving gel. mPAGE® 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 mPAGE® 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

- mPAGE® TurboMix Resolving Solution (20% acrylamide)
- mPAGE® TurboMix Stacking Solution (4% acrylamide)

Additional materials required

(not provided)

- TEMED
- 10% APS
- Gel casting system such as mPAGE® Gel Caster or equivalent with compatible glass plates and combs
- 5 mL sterile serological pipettes
- MOPS-SDS running buffer or MES-SDS running buffer
- 4X LDS Sample Buffer
- mPAGE® MES-SDS or MOPS-SDS running buffer and mPAGE® 4X LDS Sample buffer can be used with mPAGE® TurboMix

Storage

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

Bring all solutions to room temperature before use.

Important:

- Bis-Tris gels are not compatible with Tris-Glycine-SDS running buffer. Only use MOPS-SDS or MES-SDS 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.
- See page 3 to determine the volume of Resolving Solution and Stacking Solution for your gel size and thickness. Users may need to empirically determine the required volumes.

See Product Ordering on page 8 to order mPAGE® Gel Casters (sold separately)

Volumes Required To Cast One Mini Gel

Below are the solution volumes needed to cast a single 7.4 x 8.2 cm mini gel in 1.0, 0.75, or 1.5 mm thicknesses. Volumes can be multiplied to cast several gels at once. When using different sized gel cassettes, the total volume required for casting can be determined empirically by filling cassette with water.

1 mm Thick Mini Gel

Resolving Gel

Percentage	8%	10%	12%	15%
mPAGE® TurboMix Resolving solution	2.4 mL	3 mL	3.6 mL	4.5 mL
DI water	3.6 mL	3 mL	2.4 mL	1.5 mL
10% APS	30 uL	30 uL	30 uL	30 uL
TEMED	3 uL	3 uL	3 uL	3 uL
Total	**6 mL**	**6 mL**	**6 mL**	**6 mL**

Stacking Gel

Percentage	4%
mPAGE® TurboMix Stacking solution	2 µL
DI water	--
10% APS	20 µL
TEMED	2 µL
Total	**2 mL**

0.75 mm Thick Mini Gel

Resolving Gel

Percentage	8%	10%	12%	15%
mPAGE® TurboMix Resolving solution	1.8 mL	2.25 mL	2.7 mL	3.38 mL
DI water	2.7 mL	2.25 mL	1.8 mL	1.12 mL
10% APS	22.5 uL	22.5 uL	22.5 uL	22.5 uL
TEMED	2.25 uL	2.25 uL	2.25 uL	2.25 uL
Total	**4.5 mL**	**4.5 mL**	**4.5 mL**	**4.5 mL**

Stacking Gel

Percentage	4%
mPAGE® TurboMix Stacking solution	1.5 mL
DI water	--
10% APS	15 uL
TEMED	1.5 uL
Total	**1.5 mL**

1.5 mm Thick Mini Gel

Resolving Gel

Percentage	8%	10%	12%	15%
mPAGE® TurboMix Resolving solution	3.6mL	4.5 mL	5.4 mL	6.75 mL
DI water	5.4 mL	4.5 mL	3.6 mL	2.25 mL
10% APS	45 uL	45 uL	45 uL	45 uL
TEMED	4.5 uL	4.5 uL	4.5 uL	4.5 uL
Total	**9 mL**	**9 mL**	**9 mL**	**9 mL**

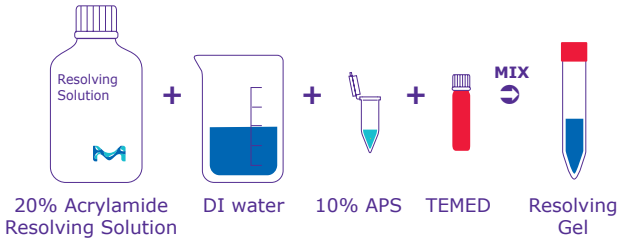
Stacking Gel

Percentage	4%
mPAGE® TurboMix Stacking solution	3 mL
DI water	--
10% APS	30 uL
TEMED	3 uL
Total	**3 mL**

Quick Cast Instructions

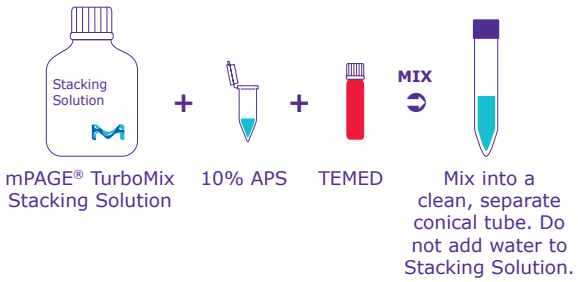
1. Bring solutions to room temperature.
2. Clean and set up casting equipment.
3. Mark desired height of resolving gel, usually 0.5-1 cm below the bottom of the comb teeth.
4. Prepare 10% APS from powder. Note: 10% APS can be prepared fresh with every use or aliquoted and stored at -20 °C. Avoid repeated freeze-thaw cycles.
5. See page 3 to calculate the volume of reagents required for the desired gel thickness.

   Multiply volumes by the desired number of gels to cast multiple gels at once (up to 4 gels).
6. Prepare the desired resolving gel percentage by pipetting into a clean conical tube:

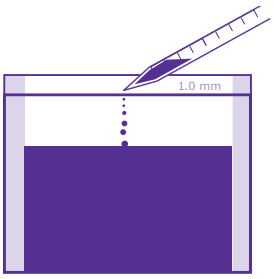


   To avoid contamination, use a sterile serological pipette to draw mPAGE® TurboMix solutions from stock bottles.
   NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.
7. Gently mix reagents by inverting conical tube, avoiding introduction of air bubbles into the gel mixture.
8. Prepare the stacking gel by pipetting mPAGE® TurboMix Stacking Solution into a clean, separate conical tube and adding the required amount of TEMED and 10% APS.

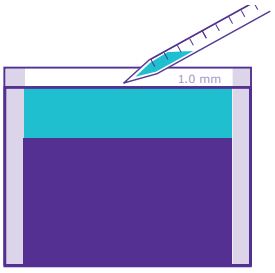
   Note: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.



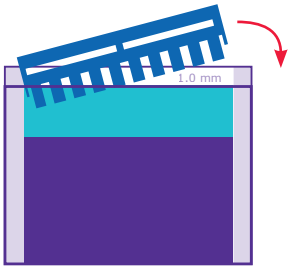
9. Gently mix reagents by inverting conical tube, avoiding introduction of air bubbles into the gel mixture.
10. Using a serological pipette, fill each cassette to marked height with resolving gel.



11. 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.



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. Left over casting solution can be used to monitor polymerization.

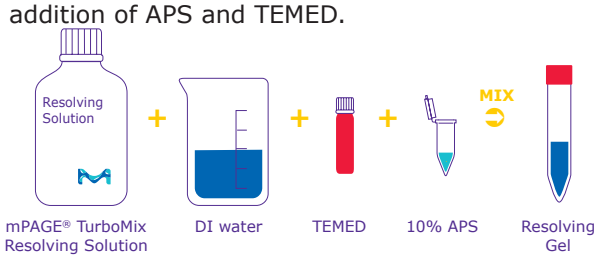


14. Gels can be used immediately or wrapped in DI water-soaked paper towels and stored in an air-tight container at 4 °C for up to 4 weeks.

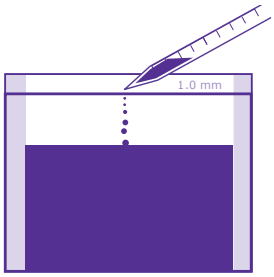
Traditional Cast Instructions

1. Bring solutions to room temperature.
2. Clean and set up casting equipment.
3. Mark desired height of resolving gel, usually 0.5-1 cm below the bottom of the comb teeth.
4. 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.
5. See page 3 to calculate the volume of reagents required for the desired gel thickness. Multiply volumes by the desired number of gels to cast multiple gels at once.
6. Prepare the desired resolving gel percentage by pipetting mPAGE® TurboMix Resolving Solution, DI water, APS and TEMED into a clean conical tube. To avoid contamination, use a sterile serological pipette to draw mPAGE® TurboMix solutions from stock bottles.

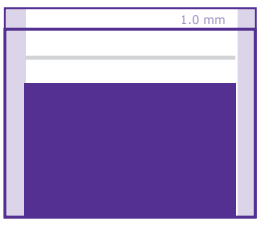
   NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.



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

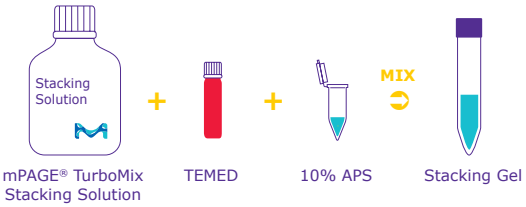


8. Using a serological pipette, fill each cassette to marked height with resolving gel.
9. Carefully overlay with isopropanol. Allow resolving gel to polymerize, approximately 30 minutes. A sharp line will appear below the isopropanol as acrylamide polymerizes. Alternatively, left over casting solution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

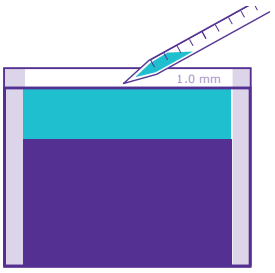


10. Remove the isopropanol and rinse with DI water. Blot with filter paper to remove residual water.
11. To prepare the stacking gel, pipette the required amount of mPAGE® TurboMix Stacking Solution, TEMED and 10% APS into a clean conical.

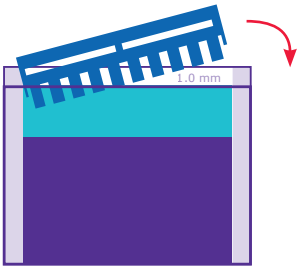
    NOTE: Add 10% APS and TEMED immediately before casting. Gel will begin to polymerize after addition of APS and TEMED.



12. 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.



13. Quickly and carefully insert the comb. Inserting at an angle may help to avoid trapping air bubbles below the teeth.
14. Allow gels to polymerize for 1 hour.



15. 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 the table below.

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

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

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 a maximum of 200 V when using MOPS running buffer, and a maximum of 180 V when using MES running buffer.

Buffer Formulation

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

4X LDS Sample Buffer

(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

(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

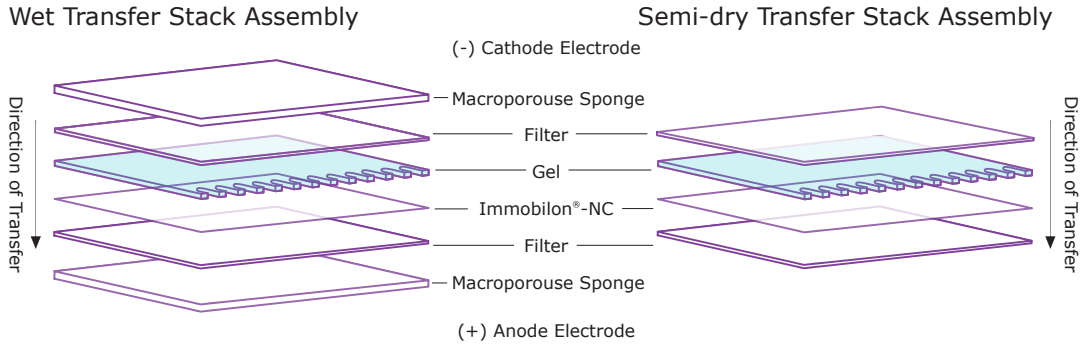
(MPMES)

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

Protein Transfer Methods

Proteins can be transferred to PVDF (polyvinylidene difluoride) or nitrocellulose (NC) blotting membranes following protein separation. The membrane type used in the application may need to be determined experimentally based on the protein characteristics. It is recommended to use a low fluorescent blotting membrane such as the Immobilon FL when fluorescent detection is desired. Proteins can be transferred onto the blotting membrane by wet transfer, also known as tank transfer, semi-dry transfer, or fast semi-dry transfer.

Prepare fresh transfer buffer based on the type of transfer to be performed. For compatible buffer please refer to the table below. Depending on the protein of interest, choice of blotting membrane and transfer system used, optimization of the alcohol percent and transfer conditions may be required.



	Wet transfer	Semi-Dry Transfer	Fast Semi-Dry Transfer
Transfer system	mPAGE® Mini Wet Transfer System	Trans-Blot® SD Semi-Dry Transfer Cell (Bio-Rad)	Trans-Blot® Turbo™ Transfer System (Bio-Rad)
Transfer condition	100V, 60 minutes	25V, 30 minutes	1.3 A, 25V limit, 7 minutes
Gel incubation prior to transfer	Optional	5-10 minutes	No gel incubation
Filter paper	Immobilon® Blotting Filter Paper (IBFP0785C)	BioRad (1703966)	Part of transfer kit
Compatible Transfer buffer	1X mPAGE® Transfer Buffer with 10% or 20% methanol	2X mPAGE® Transfer Buffer with 10% methanol	Proprietary transfer buffer only available as part of a kit
	Tris-Glycine Buffer, pH 8.3 with 20% methanol	Tris-Glycine Buffer, pH 8.3 with 20% methanol	
	Bjerrum Schafer-Nielsen Buffer with 20% methanol	Tris-Glycine Buffer, pH 8.3 with 20% methanol, 0.025% SDS (best for high MW proteins)	
		Bjerrum Schafer-Nielsen Buffer with 20% methanol	

Product Ordering

Purchase online at SigmaAldrich.com.

Description	Qty/Pk	Catalogue Number
mPAGE TurboMix Bis-Tris Gel Casting		
mPAGE® TurboMix Bis-Tris Gel Casting Kit	~10 mini gels	TMKIT-10
mPAGE® TurboMix Bis-Tris Gel Casting Kit	~60 mini gels	TMKIT-60
mPAGE® TurboMix Resolving Solution	216 mL	TMRES-216ML
mPAGE® TurboMix Stacking Solution	120 mL	TMSTK-120ML

Gel Caster and Kits

mPAGE® Gel Caster (2 pk) Contains: Gel Caster Base (2), Gel Caster Frame (2), Gel Caster Sealing Gaskets (2), Gel Releasers (5)	1	GCR2
mPAGE® Gel Casting Kit, 0.75 mm Contains: Gel Caster (1), Combs 10 wells (5), Combs 15 wells (5), Mini Spacer Plates (5), Mini Short Plates (10)	1	MGCK-75M
mPAGE® Gel Casting Kit, 1.0 mm Contains: Gel Caster (1), Combs 10 wells (5), Combs 15 wells (5), Mini Spacer Plates (5), Mini Short Plates (10)	1	MGCK-10M
mPAGE® Gel Casting Kit, 1.5 mm Contains: Gel Caster (1), Combs 10 wells (5), Combs 15 wells (5), Mini Spacer Plates (5), Mini Short Plates (10)	1	MGCK-15M
mPAGE® Combs		
0.75 mm, 10 wells	5	C75M10W
0.75 mm, 15 wells	5	C75M15W
1.0 mm, 10 wells	5	C1M10W
1.0 mm, 15 wells	5	C1M15W
1.5 mm, 10 wells	5	C15M10W
1.5 mm, 15 wells	5	C15M15W
mPAGE® Mini Spacer Plates		
0.75 mm	5	MSPA75
1.0 mm	5	MSPA10
1.5 mm	5	MSPA15
mPAGE® Mini Short Plates	10	MSHRT

Description	Qty/Pk	Catalogue Number
mPAGE® Gel Caster Base	1	GCBASE
mPAGE® Gel Caster Frame	1	GCFRM
mPAGE® Gel Caster Sealing Gaskets	5	GCSEAL
mPAGE® Gel Releasers	5	GREL5
mPAGE® Casting Plate Rack	1	CPS1

Buffers

mPAGE™ 4X LDS Sample Buffer	10 mL	MPSB-10ML
	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

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
Lithium dodecyl sulfate (LDS)		L9781
Sodium dodecyl sulfate (SDS)		L3771
Ethylenediaminetetraacetic acid (EDTA)		E5134
Ethylenediaminetetraacetic acid (MOPS)		M1254
2-Morpholinoethanesulfonic acid monohydrate (MES)		M3671
SDS-PAGE and Transfer Systems		
mPAGE® Mini Gel Tank, makes 2 gels	1	MGT-2
mPAGE® Mini Gel Tank, makes 4 gels	1	MGT-4
mPAGE® Mini Wet Transfer System	1	MWTS

Description	Qty/Pk	Catalogue Number
Transfer Membranes		
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
Immobilon®-NC Transfer Membrane, 0.45 µm, 8.5 cm x 10 m	1 roll	HATF85R
Immobilon®-NC Transfer Membrane, 0.45 µm, 7 cm x 8.4 cm sheet	50 sheets	HATF07850
Power Supplies		
mA400 Basic Power Supply	US plug	MA400-US
	Euro plug	MA400-EU
	UK plug	MA400-UK
	Japan plug	MA400-NI
	China plug	MA400-ZH
mA700 Essential Power Supply	US plug	MA700-US
	Euro plug	MA700-EU
	UK plug	MA700-UK
	Japan plug	MA700-NI
	China plug	MA700-ZH

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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