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Application Note

Rapid Visualization of In-Gel Protein Detection Without Staining Using the mPAGE® Lux Casting System

INTRODUCTION

SDS-PAGE is a common technique used to separate proteins and other biomolecules by size. Proteins within the gel can be detected using well-established colorimetric or fluorescent stains, like Coomassie Brilliant Blue (CBB), Silver Stain, and SYPRO™ Ruby. These staining methods vary in sensitivity and often require several time-consuming incubation steps.

First described as a fluorescent quencher, trichloroethanol (TCE) is a compound that shifts tryptophan fluorescence from its native emission (~350 nm) towards visible light (~512 nm) by producing a new fluorophore upon activation with UV light.[1-3] This property of TCE and other trichloro compounds was exploited by Kazmin et. al. to develop a fast and sensitive one-step gel staining solution.[4] Later, TCE was incorporated directly into SDS-PAGE gels during gel preparation which allowed detection of proteins without cumbersome staining steps.[5,6] Here we describe a protocol for preparing mPAGE® Lux gels with TCE to allow for in-gel protein detection without staining.

METHODS

Materials Required

- mPAGE® Lux Bis-Tris Reagent Kit (**Cat. No. LUXRGTKIT**) or individual mPAGE® Lux Reagents (**Cat. Nos. LUXRGTRES, LUXRGTDIL**, and **LUXRGTSTK**)
- mPAGE® Lux Bis-Tris Gel Casting System (**Cat. No. LUXCSYS**)
- 2,2,2-Trichloroethanol (**Cat. No.T54801-100G**)
- Pipette, pipette tips
- Serological pipettes
- Gel electrophoresis system compatible with mPAGE® Lux gels
- Gel documentation system with 302 nm transilluminator

Solution Preparation

All solutions must be protected from sunlight to prevent polymerization. Before casting gels, bring all solutions to room temperature.

Resolving Gel:

1. Prepare Resolving Gel solution by mixing mPAGE® Lux Resolving Solution, mPAGE® Lux Diluent, and TCE. See **Tables 1-3** for mixing volumes. Several gels can be prepared at once by multiplying the volumes by desired number of gels.
2. Add Resolving Solution to a black mixing tube or other opaque container.
3. Add Diluent to the same mixing tube.
4. Add TCE to the same mixing tube. The final TCE concentration is 1%. Do not add TCE to the original Resolving Solution or Diluent bottles.

Gel Percentage	Resolving Solution		Diluent		TCE Volume		Total Volume
8%	1.5 mL	+	2.2 mL	+	37.5 µL	=	3.7 mL
10%	1.9 mL	+	1.8 mL	+	37.5 µL	=	3.7 mL
12%	2.2 mL	+	1.5 mL	+	37.5 µL	=	3.7 mL
13.5%	2.5 mL	+	1.2 mL	+	37.5 µL	=	3.7 mL

Table 1. *0.75 mm* Solution Volumes.

Gel Percentage	Resolving Solution		Diluent		TCE Volume		Total Volume
8%	2 mL	+	3 mL	+	50 µL	=	5 mL
10%	2.5 mL	+	2.5 mL	+	50 µL	=	5 mL
12%	3 mL	+	2 mL	+	50 µL	=	5 mL
13.5%	3.3 mL	+	1.7 mL	+	50 µL	=	5 mL

Table 2. *1.0 mm* Solution Volumes.

Gel Percentage	Resolving Solution		Diluent		TCE Volume		Total Volume
8%	3 mL	+	4.5 mL	+	75 µL	=	7.5 mL
10%	3.75 mL	+	3.75 mL	+	75 µL	=	7.5 mL
12%	4.5 mL	+	3 mL	+	75 µL	=	7.5 mL
13.5%	5 mL	+	2.5 mL	+	75 µL	=	7.5 mL

Table 3. *1.5 mm* Solution Volumes.

Stacking Gel:

The mPAGE® Lux Stacking Solution is ready to use, do not dilute.

- For 0.75 mm gels, use 1.1 mL Stacking Solution
- For 1.0 mm gels, use 1.5 mL Stacking Solution
- For 1.5 mm gels, use 2.25 mL Stacking Solution

Gel Casting

1. From the Ready screen, select gel thickness by pressing 0.75 mm, 1.00 mm, or 1.50 mm button on the mPAGE® Lux Gel Curing Station display.
2. Open the mPAGE® Lux Curing Station door and insert assembled mPAGE® Gel Caster into mPAGE® Lux Curing Station by placing the front edge of the caster behind the left and right alignment bumpers.
3. Using a clean serological pipette, add prepared Resolving Gel Solution to the indicated fill line. Position the pipette in the center of the glass when filling the cassette.
4. Using a clean serological pipette, slowly add Stacking Solution to the top of the short plate. Position the pipette in the center of the glass when filling the cassette. A dip may occur but will level out.
5. Insert comb into cassette at an angle to help prevent bubble entrapment under comb teeth.
6. Wipe away any acrylamide spill-over onto the short plate. With the hooks towards the back, firmly push the mPAGE® Clip-on Mask down over glass cassette and back of caster frame. The front of the mask should sit snuggly against the short plate, covering the comb teeth completely. The mPAGE® Clip-on Mask prevents the gel from curing around the comb.
   a. **Note**: Avoid pushing on the front of the plates while installing the mPAGE® Clip-on Mask, this may cause the gel to seep out.
7. Close mPAGE® Lux Curing Station door. Ensure door is completely closed before proceeding to gel curing.
8. Press Start/Cancel button to begin gel curing. A timer will display time remaining. When curing is complete, a beep will sound, and the screen will flash COMPLETE.
9. Once curing is complete, remove gel and use. Gel can be used immediately.
10. To make more gels, use a 2nd mPAGE® Gel Caster for doing steps 3-6 outside of the mPAGE® Lux Curing Station. Carefully place them into the mPAGE® Lux Curing Station and do steps 7 and 8.

Electrophoresis

The gels prepared with the mPAGE® Gel Caster are compatible with the mPAGE® Mini Gel Tank or Bio-Rad Mini-PROTEAN® Cells. Gels should be run with MOPS-SDS or MES-SDS running buffer. Do not use Tris-Glycine running buffer. Gels can be run at up to 200 V, depending on the gel thickness, running buffer, and number of gels per tank.

1. Prepare samples with LDS buffer and DTT.
2. Heat samples for 10 minutes at 70 °C then briefly centrifuge. Do not boil samples.
3. For best results, remove comb before installing gel in electrophoresis tank. To remove the comb, firmly and evenly pull up both sides of the comb from the gel.
4. Install gels into the electrophoresis core and place inside tank. Add 1X MOPS-SDS or 1X MES-SDS running buffer.
5. Rinse wells with running buffer gently to remove any debris.
6. Load samples and protein standard(s) using gel loading pipette tip.
7. Run gels until dye front reaches the bottom of the gel or until proteins have achieved adequate separation.

    a. For MOPS-SDS running buffer, run gels at 200 V.
    b. For MES-SDS running buffer, run gels at 180 V.
    c. **Note**: 1.5 mm gels should be run at 150 V in MOPS-SDS running buffer or 120 V in MES-SDS running buffer to minimize heat generation and obtain sharper bands.

Protein Visualization

1. After electrophoresis, remove gel from glass cassette by cutting along the sides. Remove stacker and wells by cutting it away from the resolving gel. Carefully rinse gel with water.
2. Acquire images by activating the gel on UV setting for 45 seconds using gel documentation system, then capture fluorescent images by exposing for 2-20 seconds, depending on band brightness.
3. After imaging, gels can be processed with other staining methods if desired.

RESULTS

TCE Titration

Purified proteins loaded with increasing TCE concentrations between 0-2% were detected. The signal intensity peaked at 1% TCE, with no signal intensity changes after further concentration increases (**Figure 1**). Two different activation times were tested (5 minutes and 45 seconds), with similar sensitivity of detection observed for both times. They both were able to detect as little as 15 – 60 ng of protein.

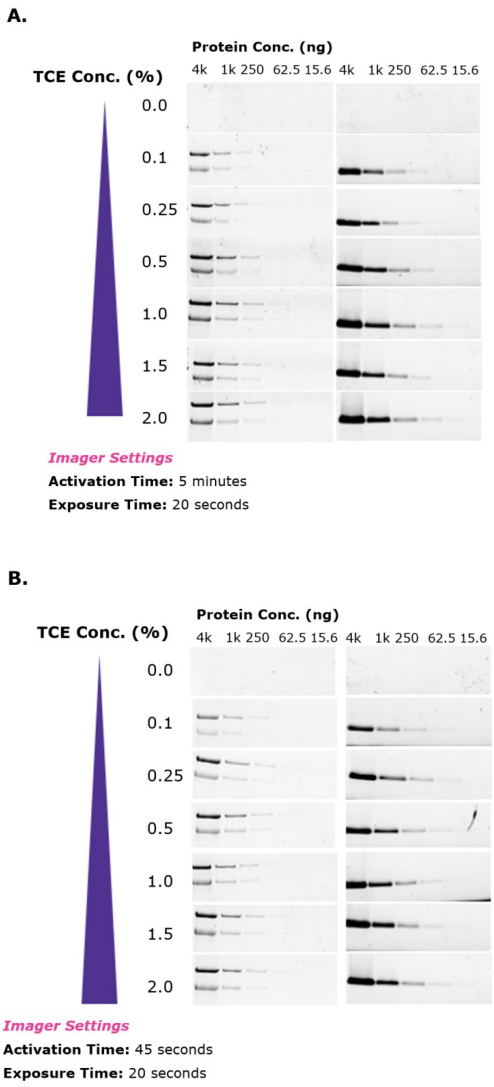

Figure 1. Titration of TCE in mPAGE® Lux Gels. Protein detection on 12% mPAGE® Lux Bis-Tris gels with increasing concentrations of TCE (0-2%) added. Three purified proteins (Lysozyme (14.4 kDa), BSA (66 kDa), Phosphorylase b (97 kDa)) are loaded with 4 µg, 1 µg, 0.25 µg, 0.0625 µg, 0.0156 µg, 0.004 µg, 0.0009 µg, and 0 µg respectively. Gels were run for 42 minutes at 200 V in MOPS-SDS running buffer. Activation time is (**A**) 5 minutes or (**B**) 45 seconds and Exposure time is 20 seconds for all gels. Maximum signal intensity is observed at 1% TCE; higher TCE % shows no further increase.

Staining vs Stain-Less Method Comparison

Comparison between TCE visualization and Coomassie gel stain methods was performed. The sensitivity of detection was similar between the two methods (**Figure 2**). However, the TCE-based detection does not require the staining step, saving at least two to several hours of prep time, depending on your current staining method.

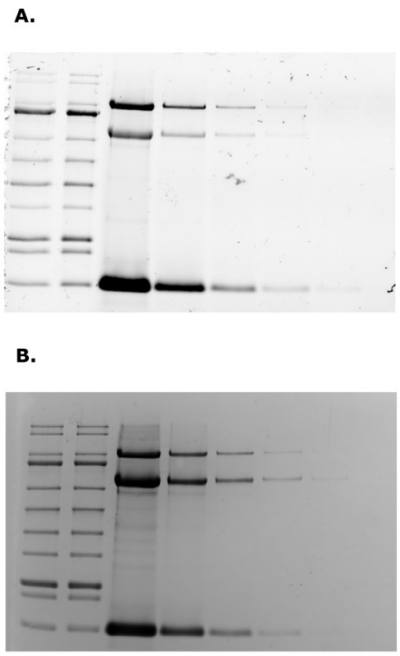

Figure 2. **Protein Visualization with TCE Compared to READYBLUE® Coomassie Gel Stain**. Three purified proteins (Lysozyme (14.4 kDa), BSA (66 kDa), Phosphorylase b (97 kDa)) are loaded with 4 µg, 1 µg, 0.25 µg, 0.0625 µg, 0.0156 µg, 0.004 µg, 0.0009 µg, and 0 µg respectively in a 12% mPAGE® Lux gel prepared with 1% TCE. The gel was run for 42 minutes at 200 V in MOPS-SDS running buffer. (**A**) Protein visualization after 45 seconds of TCE activation. (**B**) Corresponding READYBLUE® Coomassie stained gel image. Similar protein detection sensitivity is observed between TCE-induced and Coomassie stained gel.

Method Compatibility

Compatibility of this method with downstream analysis, in this case Western blotting, was also performed. **Figure 3** shows a comparison of a blot prepared with and without TCE. Similar immunodetection sensitivity was observed when the gels were activated for 45 seconds and imaged prior to transfer, as shown here.

However, due to the nature of mPAGE® Lux gel polymerization, longer activation and exposure times can alter protein transfer efficiency. Protein transfer may need to be optimized. If Western blotting will be performed after TCE visualization, it is recommended to use the shortest activation time possible because the activation can affect the transfer efficiency of the mPAGE® Lux gels.

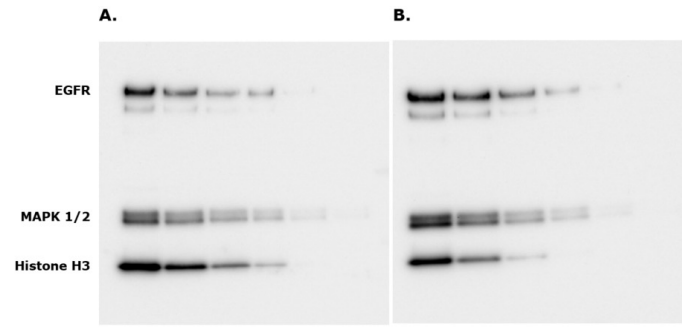

Figure 3. **Western Blot After TCE Visualization**. A 2x dilution series of EGF-stimulated A431 cell lysate (10 ng – 0.3 ng) was loaded into an 8% mPAGE® Lux gel prepared (**B**) with or (**A**) without 1% TCE. The gel was run for 38 minutes at 200 V in MOPS-SDS running buffer, then the gel containing TCE was activated for 45 seconds and imaged. Both gels were then transferred to an Immobilon®-P PVDF membrane. The membranes were probed for EFGR, MAPK 1/2, and Histone-H3 using standard immunodetection methods.

DISCUSSION

In this application note we have demonstrated that including TCE in gels is a quick and easy way to visualize proteins. Because this method does not require an extra staining step, it saves time and can be done immediately after electrophoresis. The results here also show that the sensitivity of protein detection is similar to other conventional methods that use staining.

Overall, using mPAGE® Lux gels and other mPAGE® electrophoresis products, you can quickly and reproducibly make and run gels. In addition, mPAGE® Lux gels are compatible with the stain-less technology described here using TCE, so it further reduces steps in your current protocols. This provides researchers with a valuable tool to still rely on the ease and flexibility of mPAGE® Lux casting technology, while now also getting the added benefit of rapid protein visualization.

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ORDERING INFORMATION

Casting System

Product Name	Cat. No.
mPAGE® Lux Casting System, 1 mm	**LUXCSYS-1M**
mPAGE® Lux Casting System, 0.75 mm	**LUXCSYS-75M**
mPAGE® Lux Casting System, 1.5 mm	**LUXCSYS-15M**

Reagents

Product Name	Cat. No.
2,2,2-Trichloroethanol (TCE)	**T54801**
mPAGE® Lux Bis-Tris Reagent Kit (contains 3 mPAGE® reagents below)	**LUXRGTKIT**
mPAGE® Lux Bis-Tris Resolving Solution for mPAGE® Lux Curing Station	**LUXRGTRES**
mPAGE® Lux Bis-Tris Diluent for mPAGE® Lux Curing Station	**LUXRGTDIL**
mPAGE® Lux Bis-Tris Stacking Solution for mPAGE® Lux Curing Station	**LUXRGTSTK**

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mPAGE® Lux Clip-on Mask	**LUXCLP**
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mPAGE® Unstained Protein Standard	**MPSTD3**
mPAGE® Western Protein Standard	**MPSTD2**
Transfer Buffer Powder for use with mPAGE® Bis-Tris Gels	**MPTRB**
Immobilon®-P PVDF Membrane	**IPVH00010**
MOPS-SDS Running Buffer Powder for mPAGE® Bis-Tris Gels	**MPM0PS**
MES-SDS Running Buffer Powder for mPAGE® Bis-Tris Gels	**MPMES**
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mA700 Essential Power Supply	**MA700**
SNAP i.d.® 2.0 Protein Detection System	**SNAP2MIDI**

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