INSTRUCTIONS

MemCode™ Reversible Protein Stain Kit – for PVDF Membranes

24585

1485.1

Number	Description
24585	**MemCode Reversible Protein Stain Kit – for Polyvinylidine difluoride (PVDF) Membranes,** sufficient material for 10 (8 cm × 8 cm) PVDF membranes

Kit Contents:

MemCode™ Sensitizer, 250 ml

MemCode™ Reversible Stain, 250 ml

MemCode™ Destain, 1,000 ml

MemCode™ Stain Eraser, 250 ml

Storage: Upon receipt store kit at room temperature.

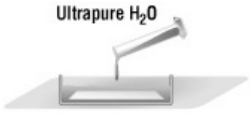
Introduction

Thermo Scientific MemCode Reversible Protein Stain is a sensitive method for staining proteins on PVDF membranes to confirm the efficiency of protein transfer. The staining method is simple, quick and results in turquoise-blue bands that do not fade and are easily photographed for future reference. The stain can be easily reversed in less than 15 minutes. Subsequent Western blot detection is unaffected because the stain is completely removed and does not alter the protein.

Other protein staining systems available for PVDF membranes such as coomassie dye and Ponceau S have disadvantages. Ponceau S is less sensitive than other available stains and results in red protein bands that easily fade and are difficult to photograph. Coomassie dye is a sensitive stain, but it permanently binds to proteins and can interfere with Western blotting.

MemCode Reversible Protein Stain uses a stain that has a high affinity for protein but does not permanently bind. The stain has minimal nonspecific interactions with PVDF and protein transfer reagents and is compatible with general Western blot systems. The treated membrane does not interfere with conventional chemiluminescent or chromogenic detection using HRP and alkaline phosphatase substrates. The stain is also compatible with N-terminal sequence analysis of proteins. This kit provides an easy, reliable and sensitive method to stain proteins on PVDF membranes before proceeding to either the Western blot analysis or N-terminal sequence analysis.

Procedure Summary

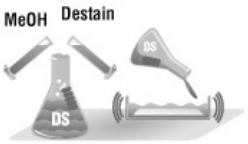


1. Wash membrane with ultrapure water.

2. Add MemCode™ Sensitizer. Shake for 2 minutes.

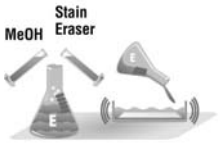
3. Add MemCode™ Stain. Shake for 1 minute.

4. Rinse three times with MemCode™ Destain.



5. Wash with Destain/Methanol Solution on a shaker for 5 minutes.

6. Rinse five times with ultrapure water.



7. Wash with Eraser/Methanol Solution on a shaker for 10-20 minutes.

8. Rinse five times with ultrapure water.

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Additional Materials Required

- Ultrapure water
- PVDF membrane containing transferred proteins

  Note: This product is not suitable for nitrocellulose membranes. For nirocellulose membranes, use MemCode Reversible Protein Stain Kit – for Nitrocellulose Membranes (Product No. 24580).
- Methanol (Reagent Grade)
- Rotary platform shaker for membrane agitation during incubations

Material Preparation

Destain/Methanol Solution	Mix reagent grade methanol 1:1 with MemCode™ Destain. For example, mix 12 ml reagent grade methanol with 12 ml MemCode™ Destain.
Eraser/Methanol Solution	Mix reagent grade methanol 1:1 with MemCode™ Stain Eraser. For example, mix 12 ml reagent-grade methanol with 12 ml MemCode™ Stain Eraser.

Procedure for Reversible Staining of Proteins on PVDF Membranes

Important Note: For all steps, use sufficient volumes to completely immerse the membrane. There are sufficient reagents for 10 (8 cm × 8 cm) PVDF membranes if 25 ml is used at each step. More solution may be necessary if using a large tray. Adjust volumes as required for different membrane sizes. Do not let the membrane become dry at any time during the procedure.

- **Stain**

1. Place the membrane containing the transferred proteins in a suitable container. To rinse the membrane, add ultrapure water, manually rock the container three times and quickly decant.
2. Add the MemCode™ Sensitizer to the membrane. Agitate membrane at room temperature for 2 minutes on a rotary platform shaker at moderate speed. Decant solution.
3. Add the MemCode™ Reversible Stain to the membrane. Agitate membrane at room temperature for 1 minute on a rotary platform shaker at moderate speed. Decant solution. Stained proteins appear as turquoise-blue bands.

- **Destain (remove background)**

4. Add of the MemCode™ Destain to the membrane, rock the container three times and quickly decant the solution. Repeat this step two additional times.
5. Add the Destain/Methanol Solution to the membrane. Agitate the membrane at room temperature for 5 minutes on a rotary platform shaker at moderate speed. Decant solution.
6. To rinse membrane, add ultrapure water, manually rock the container three times and quickly decant. Repeat this step four additional times.

- **Erase the stain (remove stain from bands)**

7. Add the Eraser/Methanol Solution to the membrane. Agitate membrane at room temperature for 10 minutes on a rotary platform shaker.

   Note: Ten minutes agitation is optimal for most of the proteins, but it may be extended up to 20 minutes.
8. Decant solution. To rinse the membrane, add ultrapure water, manually rock the container three times and quickly decant. Repeat this step four additional times.

Troubleshooting

Problem	Cause	Solution
Bands faint or not visible	Low amounts or no protein present in the sample	Determine the protein concentration in the original sample
Stain is not completely erased	Membrane was allowed to dry	Always keep the membrane wet
	High protein concentration in the sample	Extend the incubation in Eraser/Methanol Solution (Step 7) to 20 minutes
		Reduce the protein concentration in the sample

Related Thermo Scientific Products

24580 **MemCode™ Reversible Protein Stain Kit – for Nitrocellulose Membranes**

34075 **SuperSignal® West Dura Extended Duration Substrate,**100 ml

34080 **SuperSignal® West Pico Chemiluminescent Substrate,** 500 ml

34095 **SuperSignal® West Femto Maximum Sensitivity Substrate,** 100 ml

21059 **Restore™ Western Blot Stripping Buffer,** 500 ml

21065 **Erase-It® Background Eliminator Kit,** for eliminating background from X-ray film

24597 **Color Silver Stain Kit**

24612 **SilverSNAP® Stain Kit II**

24600 **SilverSNAP® Stain for Mass Spectrometry**

24590 **GelCode™ Blue Stain Reagent,** 500 ml

Product References

Stepan, V., *et al*. (2004). Role of small GTP binding proteins in the growth-promoting and antiapoptotic actions of gastrin. *.Amer. J. Physiol-Gastrointest L.* **287:**G715-25.

Ruehl, M., *et al*. (2005). The elongated first fibronectin type III domain of collagen XIV is an inducer of quiescence and differentiation in fibroblasts and preadipocytes. *J. Biol. Chem*. **280(46):**38537-43.

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