No-Stain™ Protein Labeling Reagent

Catalog Number A44449

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Experienced user protocols

No-Stain™ protocol for gels

Note: For Tris-Glycine gels, perform four 2-minute water washes with shaking before you begin to remove residual glycine.

Prepare the **No-Stain™ Labeling Solution** during gel electrophoresis, per gel:

- 20 mL **1X Labeling Buffer**
  - 1 mL **No-Stain™ Labeling Buffer (20X)**
  - 19 mL UltraPure™ water
- 20 µL **No-Stain™ Activator**
- 20 µL **No-Stain™ Derivatizer**

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Incubate the gel in the **No-Stain™ Labeling Solution** without shaking or agitation for 10 minutes at room temperature.

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Image the gel with a 488 nm excitation and 590 nm emission channel, such as the **No-Stain™ Labeled Gel** channel on an iBright™ FL1500 Imaging System.

No-Stain™ protocol for membranes

Note: For membranes from Tris-Glycine gels, perform two 2-minute water washes with shaking before you begin to remove residual glycine.

Prepare the **No-Stain™ Labeling Solution** during protein transfer, per mini-sized membrane (double the volumes for a midi-sized membrane):

- 10 mL **1X Labeling Buffer**
  - 0.5 mL **No-Stain™ Labeling Buffer (20X)**
  - 9.5 mL UltraPure™ water
- 20 µL **No-Stain™ Activator**
- 20 µL **No-Stain™ Derivatizer**

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Incubate the membrane in the **No-Stain™ Labeling Solution** on a shaker for 10 minutes at room temperature.

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Wash the membrane with water 3 times for 2 minutes each time.

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Image the membrane with a 488 nm excitation and 590 nm emission channel, such as the **No-Stain™ Labeled Membrane** channel on an iBright™ FL1500 Imaging System.

Product description

The No-Stain™ Protein Labeling Reagent provides an accurate, reliable method for visualizing proteins in a gel as an alternative to traditional gel stains or for visualizing proteins on a membrane (post-transfer) for performing Total Protein Normalization (TPN). The No-Stain™ reagent forms covalent bonds with the lysine residues of proteins. The labeling reaction is complete within 1 hour. However, labeling for 10 minutes is usually sufficient. No de-staining steps are needed. Gels and membranes can be instantly visualized using any commonly available imager. The No-Stain™ reagent is compatible with different gel chemistries, commonly used gel stains, and western workflows.

Contents and storage

Contents	Volume	Storage
No-Stain™ Activator	800 µL	–20°C
No-Stain™ Derivatizer	800 µL	
No-Stain™ Labeling Buffer (20X)	2 x 20 mL	15–30°C

Procedural guidelines

Guidelines for thawing

- Thaw all components to room temperature (21–25°C) before use.
- Do not heat the No-Stain™ Activator or No-Stain™ Derivatizer above 28°C. Excess heating will negatively affect the material's stability and subsequent function.
- Thoroughly mix all solutions, verifying that no particulate material is present in the bottle or tubes before use.

Guidelines for labeling

- Labeling time can be increased based on the sensitivity needs of the experiment and the amount of protein loaded.
- The No-Stain™ signal will continue to increase for >30 minutes. Labeling times can be optimized for obtaining desired sensitivity.
- The volumes of the No-Stain™ Activator and No-Stain™ Derivatizer can also be proportionately increased to increase the labeling intensity or decrease the required labeling time.

Guidelines for imaging

- The No-Stain™ fluorescent signal has an emission maximum of approximately 590 nm.
- Excite the fluorophore using a green UV light source transilluminator or blue light source.
- Capture the fluorescent signal using an appropriate emission filter.

Detailed usage protocols

Note: The solution may initially appear milky but will clarify after mixing.

IMPORTANT! It is recommended to use the prepared No-Stain™ Labeling Solution within 60 minutes of preparation.

No-Stain™ labeling of gels

1. Prepare the **No-Stain™ Labeling Solution** during gel electrophoresis, per gel:
   - 20 mL **1X Labeling Buffer**
     - 1 mL **No-Stain™ Labeling Buffer (20X)**
     - 19 mL UltraPure™ water
   - 20 µL **No-Stain™ Activator**
   - 20 µL **No-Stain™ Derivatizer**
2. Skip this step and proceed to step 3 for non-Tris-Glycine gels.

   Wash the Tris-Glycine gel in 20 mL of UltraPure™ water four times for 2 minutes each time on a platform rotating at approximately 60 rpm.
3. Add 20 mL of the prepared No-Stain™ Labeling Solution to the dish containing the gel, then incubate for 10 minutes at room temperature without shaking. Ensure that the gel is completely submerged in the No-Stain™ Labeling Solution.
4. Image the gel using an appropriate imaging instrument with the appropriate settings (see "Guidelines for imaging" on page 1). The No-Stain™ fluorescent signal has an emission maximum of approximately 590 nm.

   When labeling gels, avoid using gel sample preparation buffers (unless the dye front is being cut off the gel or has migrated off the gel) or protein ladders that generate signal when imaging using a 488/590 nm excitation/emission wavelength channel.

No-Stain™-labeled gels can be transferred to low fluorescence PVDF membranes following recommended transfer protocols. For western blotting of gels, it is recommended that low fluorescent membranes like those in iBlot™ 3 or PowerBlotter PVDF transfer stacks be used. **Note:** Water washes and No-Stain™ labeling of gels may impact the efficiency of protein transfers to membranes. Transfer of No-Stain™-labeled protein is not compatible with Tris-Glycine gels or Tris-Glycine transfer buffers, including 1-Step Transfer Buffer.

No-Stain™ labeling of membranes

1. Prepare the **No-Stain™ Labeling Solution** during protein transfer, per mini-sized membrane (double volumes for a midi-sized membrane).
   - 10 mL **1X Labeling Buffer**
     - 0.5 mL **No-Stain™ Labeling Buffer (20X)**
     - 9.5 mL UltraPure™ water
   - 20 µL **No-Stain™ Activator**
   - 20 µL **No-Stain™ Derivatizer**
2. Skip this step and proceed to step 3 for membranes transferred from non-Tris-Glycine gels.

   Wash the membrane in 20 mL of ultrapure water two times for 2 minutes each on a platform rotating at approximately 60 rpm.
3. Add 10 mL of the prepared No-Stain™ Labeling Solution to the dish containing a washed mini-sized membrane. Add 20 mL for a midi-sized membrane. Incubate the membrane in the No-Stain™ Labeling Solution for 10 minutes on a platform rotating at approximately 60 rpm.
4. Discard the No-Stain™ Labeling Solution. Wash the No-Stain™-labeled mini-sized membrane in 20 mL of UltraPure™ water 3 times for 2 minutes each time on a platform rotating at approximately 60 rpm. Discard the final wash solution and replace with another 20 mL of UltraPure™ water. Double the wash volumes for midi-sized membranes.
5. Image the membrane using an appropriate imaging instrument with the appropriate settings (see "Guidelines for imaging" on page 1). Image only the membrane (i.e., without any plastic under or on top of the membrane).

If performing an immunoassay, proceed directly to the membrane blocking step. When imaging with either fluorescence or chemiluminescence detection, the No-Stain™ signal will be present for Total Protein Normalization (TPN).

Troubleshooting

Observation	Possible cause	Recommended action
No or low fluorescent signal	Insufficient protein was loaded in gel.	Load gel with more protein.
	No lysine residues were in protein.	Load gel with lysine-containing protein.
	Gel was not submerged in Labeling Solution.	Use a flat-bottom dish with width and length dimensions approximately the same as the gel being labeled in 20 mL for a mini gel.
	Labeling reaction time was too short.	Ensure gel is incubated in Reagent at least 10 minutes up to 30 minutes.
	Incorrect or incomplete labeling reaction components were present.	Verify components and volumes are correct for Gel Labeling, not Membrane Labeling
		Ensure appropriate kit components are used.
	Imaging occurred at incorrect wavelengths.	Image with blue light (wavelength of approximately 488 nm) excitation and an emission light filter wavelength of approximately 590 nm.
	Imaging exposure time was too short.	Expose for longer times using manual exposure settings.
Dye front shows excessive signal	Dye in Sample Buffer produced signal at the same imaging channel as No-Stain™ reagent.	Switch to a fluorescent compatible sample buffer (Cat. No. LC2570).
		Cut off the lower part of the gel that contains the dye front before imaging.
		Choose a region of interest (ROI) in the imaging software to exclude the lower part of the gel.
Protein ladder bands show excessive signal	Protein ladder bands produced signal at same imaging channel as No-Stain™ reagent.	Use an unstained protein standard (Cat. No. LC5677).
		Cut off the part of the gel that contains the ladder before imaging.
		Choose a region of interest (ROI) in the imaging software to exclude the ladder.

Observation	Possible cause	Recommended action
High fluorescence signal from membrane	Membrane produced signal at the same imaging channel as No-Stain™ reagent.	Use a low-fluorescent PVDF transfer membrane (Cat. No. 22860).
Labeling solution turns pink after adding to gel	Gel components reacted with the Labeling Solution.	Perform additional water washes of the gel before adding the Labeling Solution.
Bubbles present when imaging	Air bubbles were trapped between the gel or membrane and the imager platform.	Use a Western Blot Roller (Cat. No. 84747) to remove the bubbles.
	Bubbles appeared on the surface of the gel or membrane.	Use a Western Blot Roller (Cat. No. 84747) to remove the bubbles.
		Perform a quick rinse with water of the gel or membrane.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0018742 B.0

Revision	Date	Description
B.0	29 March 2023	Reformatting with new protocols and updating to current template.
A.0	20 June 2019	New document for No-Stain Labeling Reagent User Guide.

The information in this guide is subject to change without notice.