No-Stain™ Protein Labeling Reagent

Catalog Numbers A44449

Pub. No. MAN0018742 **Rev.** A.0

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**.

CAUTION! Some materials used in this procedure are considered hazardous. The Tris contained in the No-Stain™ Labeling Buffer is an irritant. The mandelonitrile contained within the No-Stain™ Activator and the dimethylsulfoxide (DMSO) used in formulating the No-Stain™ Activator and the No-Stain™ Derivatizer are toxic. Avoid inhalation and contact with skin and eyes.

Product description

The No-Stain™ Protein Labeling Reagent provides an accurate, reliable method to visualize proteins in a gel or on a membrane (post-transfer) and perform total protein normalization. The No-Stain™ reagent forms covalent bonds to proteins in gels or on membranes by way of a reaction that is complete within 10 minutes. With no destaining steps required, gels and membranes can be instantly visualized using any commonly available imager. Furthermore, the reagent does not require any particular gel or other reagents and is compatible with gel stains and western workflows.

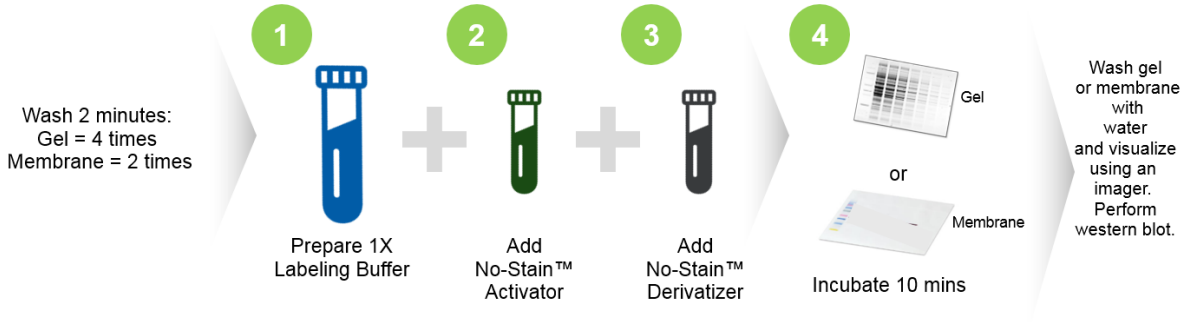

Fig. 1 No-Stain™ labeling workflow.

Contents and storage

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

Prepare solutions

Thaw all components to room temperature (21-25°C) before use. A temperature-controlled water bath set to 25°C is recommended. Do not heat the No-Stain™ Activator or No-Stain™ Derivatizer above 28°C. Excess heating will negatively impact the material's stability and subsequent function. Thoroughly mix all solutions and verify that no particulate material is present in the bottle or tubes prior to use.

1. No-Stain™ Labeling Buffer

- Dilute the provided No-Stain™ Labeling Buffer (20X) to 1X for use in subsequent preparation of the No-Stain™ Labeling Solutions. Add 1 mL of the provided No-Stain™ Labeling Buffer (20X) to 19 mL of ultrapure water in an appropriate container. Thoroughly mix the 1X buffer.
  - For labeling a **mini gel**, use 20 mL of the 1X No-Stain™ Labeling Buffer.
  - For labeling a **mini membrane**, use 10 mL of the 1X No-Stain™ Labeling Buffer. Double the volume for a **midi membrane**.

2. No-Stain™ Labeling Solution

- Prepare the No-Stain™ Labeling Solution by sequentially adding the No-Stain™ Activator, followed by the No-Stain™ Derivatizer, to the 1X No-Stain™ Labeling Buffer (from the No-Stain™ Labeling Buffer section above).

  Note: The solution may initially appear milky upon addition of the No-Stain™ Activator or Derivatizer; however, the solution will clarify after mixing.
- Mix the No-Stain™ Labeling Solution thoroughly in a tube prior to addition to the dish containing either the gel or membrane to be labeled.
- Use the prepared No-Stain Labeling Solution within 60 minutes of preparation.
  - **No-Stain™ Gel Labeling Solution**: For labeling one mini gel, add 20 µL of the No-Stain Activator, followed by 20 µL of the No-Stain™ Derivatizer, to 20 mL of the 1X No-Stain™ Labeling Buffer.
  - **No-Stain™ Membrane Labeling Solution**: For labeling one mini membrane, add 20 µL of the No-Stain™ Activator, followed by 20 µL of the No-Stain™ Derivatizer, to 10 mL of the 1X No-Stain™ Labeling Buffer. Double the volume of each component for a midi-sized membrane.

No-Stain™ labeling

Procedures for No-Stain™ labeling of mini gels and membranes are similar, but have some important differences:

Parameters	Membranes	Mini gels
Pre-washes required	2	4
Volume of Labeling Solution	10 mL	20 mL
Labeling platform	Shaking	Stationary

1. **Pre-wash**. Prepare the membrane or mini gel to be labeled by washing it with 20 mL of ultrapure water for 2 minutes on a rotating platform at ~60 rpm. Double the wash volumes for a midi-sized membrane.
   - **Gels**: Repeat for a total of 4 washes.
   - **Membranes**: Repeat for a total of 2 washes.
2. **Perform the No-Stain™ labeling**. Discard the final wash solution.
   - **Gels**: Add 20 mL of the prepared No-Stain™ Gel Labeling Solution (from the No-Stain™ Labeling Solution section above) to the dish containing the washed mini gel. Ensure that the gel is submerged and evenly covered with the No-Stain™ Gel Labeling Solution. **With the gel and dish stationary**, allow the labeling reaction to proceed for 10 minutes without shaking.
   - **Membranes**: Add 10 mL of the prepared No-Stain™ Membrane Labeling Solution (from the No-Stain™ Labeling Solution section above) to the dish containing the washed mini membrane. Double the volume for a midi-sized membrane. Allow the labeling reaction to proceed for 10 minutes **on a rotating platform at ~60 rpm**.
3. **Post wash**. After labeling for 10 minutes, discard the No-Stain™ Labeling Solution and wash the labeled mini gel or mini membrane with 20 mL ultrapure water for 2 minutes on a rotating platform at ~60 rpm. Repeat for a total of 3 washes. Discard the final wash solution and replace with 20 mL of ultrapure water. Double the wash volumes for midi-sized membranes.
4. **Imaging**. Image the gel or membrane using an appropriate imaging instrument. The No-Stain™ fluorescent signal has an emission maximum at ~590 nm. Excite the fluorophore using a green or UV light transilluminator or epi blue light source and capture the fluorescent signal using an appropriate emission filter.

   When imaging membranes, image only the membrane (i.e., without any plastic under or on top of the membrane). Note that after either fluorescence or chemiluminescence detection, the No-Stain™ signal will be present for total protein normalization.

Limited product warranty

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