Amersham™ QuickStain

Instructions for use

Introduction

Product code

RPN4000

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and not to be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at -15°C to -30°C.

Expiry

See outer packaging.

Components

The Cy™5 dye reagent is provided in solution, dissolved in dimethyl sulfoxide (DMSO).

The labeling buffer consists of Tris(hydroxymethyl)methylamine (TRIS) and Sodium Dodecyl Sulphate (SDS).

Description

Amersham™ QuickStain is a kit with a Cy5 fluorophore and labeling buffer for easy detection of proteins in SDS-PAGE gels and on Western Blot membranes. The ready-to-use Cy5 NHS ester and Tris labeling buffer ensure robust and consistent labeling for detection of proteins in diverse samples.

Samples are pre-labeled using Cy5 dye reagent prior to electrophoresis. This enables direct detection of pre-labeled proteins in the sample, eliminating the need for post-staining the gel. In Western Blotting (WB), Cy5 pre-labeled proteins are separated in the gel during electrophoresis and then transferred from the gel to a membrane. Pre-labeled samples can be used for normalization in WB, in which the total protein signal on the membrane is used as loading control.

Protocols

The table below lists the pre-labeling protocols recommended for SDS-PAGE and WB.

Pre-labeling protocol	Description
Quick pre-labeling	• Can be labeled in 5 minutes for qualitative analysis.
Western pre-labeling	• Can be used for labeling cell lysates, tissue extract, or purified protein samples prior WB analysis. • Cell lysates and tissue extracts are diluted in lysis buffer. • Purified proteins are diluted in labeling buffer.
Standard pre-labeling	• Label for 30 min at room temperature for minimal labeling variation across samples. • Samples are diluted 10 times with labeling buffer to make sure reproducible labeling for diverse sample types.

Important notes

- Samples containing a wide range of protein concentrations from as little as 1 ng/µL can be labeled. The exact protein concentration of the sample does not need to be known.
- For quantitative comparisons, it is important to use the same protocol, labeling time, and reaction volumes.
- If samples need to be diluted, it is recommended to dilute purified protein samples 1:10 in labeling buffer, and complex samples in their original lysis buffer.
- For reducing SDS-PAGE, add freshly prepared DTT to the loading buffer, to a final concentration 40 mM. This will also stop the labeling reaction.
- For non-reducing SDS-PAGE, omit the DTT. For quantitative applications add lysine to the loading buffer, 10 mM final concentration, to stop the labeling reaction.
- If the electrophoresis run will be performed at a later stage, store the pre-labeled samples in loading buffer at -20°C.
- Nitrocellulose and PVDF membranes with low auto-fluorescence properties are recommended in WB applications.
- Protocols can be scaled up as long as the relative proportions of the reagent volumes are kept constant.

- If the sample proteins are not compatible with SDS use an alternative labeling buffer, e. g., Tris pH 8.7.
- For WB of pure proteins, it is recommended to use the standard SDS-PAGE protocol. Dilute the Cy5 in water (1:10) prior to use to avoid signal saturation if needed.

Preparations

Step	Action
1	Take out one vial of Cy5 and one vial of labeling buffer (if needed) from the freezer.
2	Thaw the pre-labeling components completely.
3	Equilibrate the Cy5 vial to room temperature before opening to avoid moisture condensation.
4	Briefly spin down the Cy5 dye reagent liquid using a centrifuge.
5	Perform the labeling in 0.5 mL tubes.

After pre-preparation of the dye and labeling buffer, proceed to the protocol for preferred application.

Pre-labeling

Follow the steps in the sections below for different pre-labeling protocols.

Standard pre-labeling protocol, 40 µL final volume

Step	Action
1	Set the temperature of the heating block to 95ºC.
2	Dilute 2 µL of the sample by adding 17 µL labeling buffer and mix.
3	Add 1 µL of Cy5 dye reagent. Mix thoroughly by quickly vortexing.
4	Incubate at room temperature for 30 minutes. ***Note:*** *It is important to make sure that the labeling volume and time are equal for all samples.*
5	Add 20 µL of 2× Loading buffer with freshly prepared DTT.
6	Heat the samples at 95ºC for 3 minutes.
7	Spin down the samples.
8	Perform the electrophoresis according to manufacture's instruction.

Quick pre-labeling protocol, 40 µL final volume

Follow the steps as described for standard pre-labeling protocol. In step 4, incubate with Cy5 at 95°C for 3 to 5 minutes.

Western pre-labeling, 40 µL final volume

Step	Action
1	Set the temperature of the heating block to 95°C.
2	Add 2 to 19 µL cell lysate or tissue extract sample and fill up to a volume of 19 µL using original sample lysis buffer. For purified proteins dilute 1:10 in labeling buffer.
3	Add 1 µL of Cy5 dye reagent diluted 1:10 in ultra pure water. ***Note:*** *The diluted dye must be freshly prepared and used within 30 minutes.*
4	Briefly vortex to mix thoroughly. Incubate at room temperature for 30 minutes.
5	Add 20 µL of 2× Loading buffer with freshly prepared DTT (final concentration 40 mM).
6	Heat the samples at 95°C for 3 minutes.
7	Spin down the samples.
8	Perform the electrophoresis and WB procedure according to manufacturer's instruction.

Related products

For more information on products, pack size, and information on additional products, visit *cytiva.com/westernblotting*.

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