Amersham™ QuickStain

Product Specification Sheet

Code: RPN4000

Warning
For research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Before using this product, please read the instructions for safe handling, storage and disposal.

Storage
Store at -15°C to -30°C.

Expiry
See outer packaging.

Safety warnings and precautions
This product is used in conjunction with gel electrophoresis. Please follow the manufacturer's instructions relating to the handling and use of the equipment and materials.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Components
The Cy™5 dye reagent is provided in solution (dissolved in 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 following pre-labeling protocols are recommended for SDS-PAGE and WB.

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 ensure reproducible labeling for diverse sample types.

Quick pre-labeling
Label in 5 min for qualitative analysis.

Western pre-labeling
For labeling of 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.

Important notes
- Samples with a wide range of protein concentrations (1 ng/µL - 20 µg/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, same labeling time, and the same 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, 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, we recommend using the standard SDS-PAGE protocol and if needed diluting the Cy5 in water (1:10) prior to use to avoid signal saturation.

Preparations before starting pre-labeling
1. Take out one of each of the following vials from the freezer, 1 vial Cy5 and 1 vial labeling buffer (if needed).
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 microfuge tubes

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

Standard pre-labeling protocol, 40 µL final volume
1. Set the temperature of the heating block to 95°C.
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 95°C for 3 minutes.
7. Spin down the samples.
8. Perform the electrophoresis according to manufactures instruction.

Quick pre-labeling protocol, 40 µL final volume

As above but incubate with Cy5 at 95°C for 3-5 minutes (step 4).

Western pre-labeling, 40 µL final volume

1. Set the temperature of the heating block to 95°C.
2. Add 2-19 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 manufacturers instruction.

Related products

Description	Product code
Amersham ECL Plex™ Rainbow™ Marker	RPN850E
Amersham Protran™ Premium 0.2	10600004
Amersham Hybond™ P 0.45	10600023
Amersham Hybond™ LFP 0.2	10600022
Amersham ECL Plex Cy3™ GAM	PA43009
Amersham ECL Plex Cy3 GAR	28901106
Amersham ECL Plex Cy5 GAM	PA45009
Amersham ECL Plex Cy5 GAR	PA45011
Amersham ECL anti-rabbit wHRP Ab	NA934-1mL
Amersham ECL anti-mouse HRP Ab	NA931-1mL
Amersham ECL start Western blotting detection reagent	RPN3244
Amersham ECL Prime Western blotting detection reagent	RPN2232
Amersham ECL Select™ Western blotting detection reagent	RPN2235

For more product and pack size information plus additional products, please visit www.gelifesciences.com/westernblotting

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