CYTODIAGNOSTICS

PRODUCT DATA SHEET

Gold Protein Detection Kit for Membranes

Catalog Number: **SR-07-01**

Description

Cytodiagnostics Gold Protein Detection Kit for Membranes is a stabilized gold solution specifically developed to stain proteins blotted onto nitrocellulose or polyvinylidene difluoride (PVDF) membranes.

The negatively charged gold particles bind to protein bands with much higher affinity than to the membrane. Accumulation of gold particles onto the bound proteins produces dark permanent red bands, with better sensitivity (down to 1 ng, protein dependent) than Coomassie or the common Pounceu stain, and even rival's direct silver staining of PAGE-gels.

The kit is provided ready to use; no reconstitution or dilution is required. When used in conjunction with Cytodiagnostics Silver Enhancement Kit for Membranes (Cat. # SR-01-02), a further improvement (10 to 100 times) of sensitivity can be obtained.

Contents

The kit is supplied in ready-to-use 500 mL format, which is sufficient to stain 25 blots. 100 mL of 100X wash buffer is also supplied.

Storage/Stability

This product should be stored at 4°C and is stable for at least 6 months. DO NOT FREEZE.

Guideline of Use

Handling of membranes

This product is designed for use with negatively charged membranes such as nitrocellulose or PVDF.

Positively charged membranes, e.g. nylon membrane, will result in high background due to electrostatic interactions and should not be used.

Gloves and plastic forceps are required during the procedure to avoid smears on the membrane.

Overloading of proteins may also cause smears or even particle aggregation.

In general, the loading quantity of proteins per band or dot is recommended to be below 1 μg. A titration experiment of loading quantity may be required for specific proteins.

Assay procedure

All the following wash and incubation steps are performed at room temperature on a rocking shaker. It is important that the membrane is completely immersed in solution during the entire procedure.

1. Prepare at least 300ml 1X wash buffer by diluting 3ml of the 100X concentrated wash buffer supplied with 297ml of ddH_2O.
2. After blotting, wash the membrane in approximately 50 mL of 1X wash buffer with agitation for 15 min and repeat twice.
3. Rinse the membrane in distilled or deionized water for about 10 seconds. Repeat three times.
4. If staining in a tray, ensure that approximately 2 mm of gold solution is covering the membrane. For example, 20 mL of gold solution is enough for an 8 x 7 cm membrane, and 50 mL for a 15 x 15 cm membrane. Membranes can also be placed in sealed plastic bags and less gold solution is required.
5. The staining process is complete within 1 to 2 hours. Prolonged incubation, e.g. overnight, may increase assay sensitivity without causing over-staining.
6. After staining, wash the membrane in 50 mL of 1X wash buffer for 1 min and repeat twice.
    a. **Note**: The gold solution may be reused if stored in a separate clean container at 4 degrees. However, reduced staining sensitivity may occur in subsequent staining procedures.
7. Rinse with distilled or deionized water and air-dry membrane.

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8. Analyze results.

9. At this point the stained membrane can be stored at room temperature, or further enhanced using Cytodiagnostics Silver Enhancement Kit for Membranes (Cat. # SR-01-02). Example stains are shown below in figure 1.

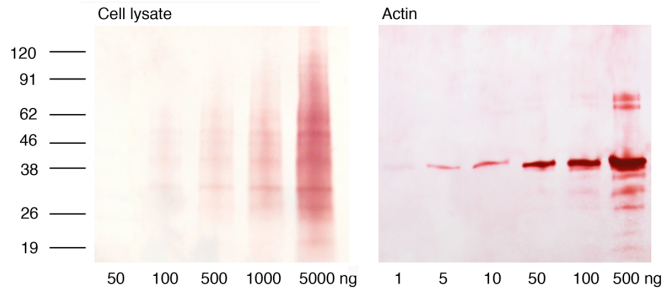

Figure 1. Cell lysate (left) and actin (right) nitrocellulose membrane stained by Cytodiagnostics Gold Protein Detection Kit for Membranes. Different amounts of cell lysate (50 ng to 5 μg) or pure actin (1 ng to 0.5 μg) were blotted following a common polyacrylamide gel electrophoresis (SDS-PAGE) running and transfer protocol, and then stained with Cytodiagnostics Gold Protein Detection Kit for Membranes.

Additional Information

Detection sensitivity

In general, our Cytodiagnostics Gold Protein Detection Kit for Membranes is capable of detecting 1 ng of protein. This sensitivity varies with the specific proteins of interest. The staining kit is optimized and supplied for the best signal-to-noise ratio for most applications.

If higher sensitivity is required, increasing the salt concentration during the stain process may improve the intensity of the protein staining. However, the background staining may also increase.

The gold nanoparticles are prone to aggregation with increased salt concentration. A titration experiment may be required to identify the optimal salt concentration for staining specific proteins. We recommend increasing the concentration of NaCl at 10 mM increments, while carefully observing staining results and potential particle aggregation*. An example for NaCl titration is listed in the table below.

To increase the concentration of NaCl for 20 mL of gold solution:

1 M of NaCl solution is prepared by dissolving 5.84 g of NaCl (Sigma #S5886) in 100 mL of water.

Final NaCl concentration (mM)	10	20	30	40	50	60	70	80	90	100
Volume of NaCl (1 M, mL)	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2

**Note: A sign of aggregating particles is a change in the color of the solution from deep wine-red to purple.*

Precautions and Disclaimer

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet available online at www.cytodiagnostics.com for information regarding hazards and safe handling procedures.

Ordering Information

For ordering call 866-344-3954 or visit us online.

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