Western Quick Block Kit and Western Quick Block Optimization Kit

Technical Manual No. 0251 **Version 05142010**

I. DESCRIPTION

GenScript Western Quick Block Kit is for quick blocking of any membranes used for Dot blot and Western blot. It can also be used for quick blocking of any solid surface such as microtiter plates used for ELISA. Instead of classical one-hour blocking of the solid surface with 5–10% dry milk, this kit enables the complete blocking of the solid surface in just 5 minutes. Furthermore, Western Quick Block Kit significantly increases the Western detection sensitivity compared with dry milk blocking. And less amount of antibodies can be used to reach the same antigen detection level as using classical blocking method.

In some cases, due to the sequence similarity between an antigen and the blocking reagents, background from Western blot (also, Dot blot and ELISA) will be so high that the antigen cannot be detected clearly.

The Western Quick Block Kit is compatible with GenScript LumiSensor™ Chemiluminescent HRP Substrate Kit (L00221V60), and ChromoSensor™ One Solution TMB Substrate (L00222V60).

II. KIT CONTENTS

Western Quick Block Kit: L00276	20 Blockings
Pretreat A	2 x 50 ml
Pretreat B	2 x 50 ml
Protocol	1

III. KEY FEATURES

- Quick blocking: The Western quick block system takes only five minutes.
- Easy to perform: simple and quick procedure for blocking.
- Sensitive: The Western quick block makes your Western detection more sensitive.

IV. STORAGE

Store the kit at 4°C (stable for six months).

V. QUICK BLOCK PROTOCOL

Materials needed but not provided:
Wash solution: PBST or any wash solution that you regularly use is fine.
Plate: GenScript provides Western blot plates for minigels. Cat. No.: M00100, M00101, M00102 and M00103.

Using Western Quick Block Kit:
This procedure is optimized for one 7.5 x 8 cm (or 10 x 10 cm) sheet of membrane. Reagent volumes may be increased or decreased in proportion to the size of membrane used.

Blocking procedure:
Just before the protein transfer from gel to membrane is complete, make quick block solution by mixing 10 ml of pretreat A solution with 10 ml of pretreat B solution in a plate. Place the membrane (no wash of the membrane after transferring is needed) in the quick block solution and incubate on a shaker for five minutes at room temperature. After incubation, rinse the membrane twice with 15 ml of wash solution (any wash solution that you regularly use is fine). Continue and complete the Western blotting procedure as usual.

Using Western Quick Block Kit for ELISA:
These two kits can also be used for quick blocking in ELISA after antigen coating. However, **do not use** the kits after primary antibody binding, because the reagents in the kits can remove the antibody from antigen.

Select the Best Pretreat A Solution
From the Western or Dot blot or ELISA results, select the pretreat A solution that gives the highest signal and lowest background (highest S/N).

Order the Customized Western Quick Block Kit
You can order the Customized Western Quick Block Kit according to the best results you obtained.

Customized Western Quick Block Kit A: L 00279	Pretreat A-a (2 x 50 ml) and pretreat B (2 x 50 ml)

VI. EXAMPLES

1. Western blot using Western Quick Block Kit (L00276)

Blocking using Western Quick Block Kit was compared with the classical 10% dry milk blocking in Western blot detection of GAPDH protein from Hela cell lysate. Serially diluted Hela cell lysate samples were Western-blotted to WestClear™ nitrocellulose membrane after SDS-PAGE. The membrane was then cut into two halves and processed with the same procedures using Goat Anti-GAPDH Polyclonal Antibody (GenScript, A00191). The only difference is using different blocking reagents: classical 10% dry milk blocking (1.0 hour, left panel of Figure 1), and using Western Quick Block Kit (5 min, right panel of Figure 1).

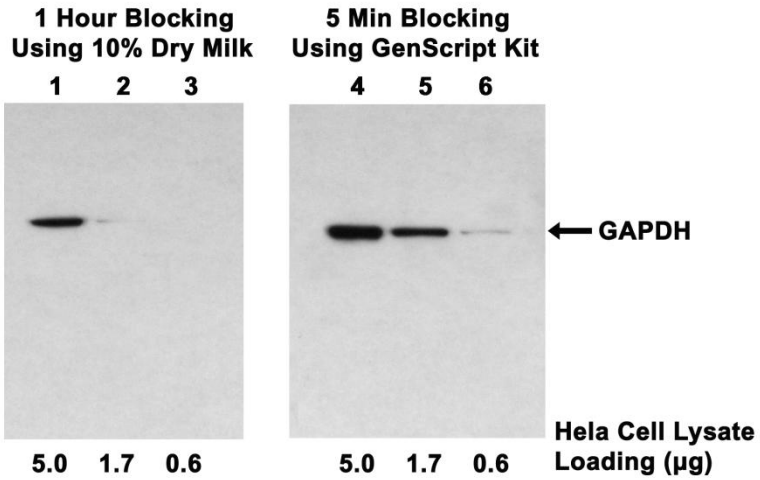

Figure 1: Western blots for the detection of GAPDH protein from Hela cell lysate by using either classical 10% dry milk Blocking or Western Quick Block Kit. 5.0 µg, 1.7 µg, and 0.6 µg of Hela cell lysate (BD Biosciences, 611449) were loaded in different lanes as shown in the figure. Both of the Western blots were developed with LumiSensor™ HRP Substrate Kit (GenScript, L00221V60).

2. Western blot using Western Quick Block Optimization Kit (L00278)

Western Quick Block Optimization Kit was used to optimize the Western blot detection of α-Tubulin in Hela cell lysate. 10 µg of Hela cell lysate was electrophoresised in 4 different lanes of SDS-PAGE and Western-blotted to WestClear™ nitrocellulose membrane. The membrane was then cut into four longitudinal strips and processed with the same procedures using unpurified monoclonal anti-α-Tubulin (Mouse Ascites Fluid, Sigma, T 5168). The only difference is using different blocking reagents as shown in Figure 2.

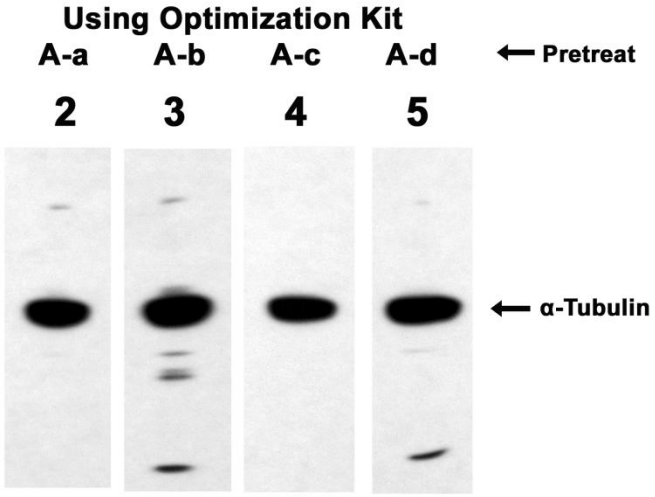

Figure 2: Western blots for the detection of α-Tubulin in Hela cell lysate by using Western Quick Block Optimization Kit. 10.0 µg of Hela cell lysate (BD Biosciences, 611449) was loaded in each of the 4 different lanes as shown in the figure. Monoclonal anti-α-Tubulin (Mouse Ascites Fluid, Sigma, T 5168) was used together with Western Optimization Kit (L00258) which contains all the components of Western Quick Block Optimization Kit.

VII. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

Problem	Probable Cause	Solution
Weak or invisible signal	Too little protein is loaded.	Load more proteins onto the SDS-PAGE gel.

	There is poor transfer efficiency.	Optimize the transfer time and /or the electrical current. Make sure that there are no air bubbles between the membrane and the gel.
	The primary antibody has a low affinity for the antigen.	Increase the incubation time of the membrane with primary antibody.
High background	Too much primary antibody or secondary is used.	Reduce the amount of antibody.
	The wash time is too short.	Wash the membrane longer or add one or two more wash steps.
	There is cross-reaction between antibodies and blocking reagent.	Use the Western Quick Block Optimization Kit to find the best blocking reagent.
	The equipment or reagents have become contaminated.	Use a clean container for each rinse and wash step. Wear gloves and use clean forceps to handle membranes.

VIII. ORDER INFORMATION

Western Quick Block Kit: Cat. No. L00276

For Research Use Only.

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