UltraBio™ Western Blot Buffer Kit

W751600

Storage temperature: Store at 4℃ for one year. Store at -20℃ for long time.

Introduction

The UltraBio™ Western Solution Kit (including Blocking Buffer, Washing Buffer, Primary Antibody Diluent, and Secondary Antibody Diluent) is a set containing the latest generation of fast and high-efficiency solutions commonly used in Western Blot experiments. The Blocking Buffer, Primary Antibody Diluent, and Secondary Antibody Diluent outperform traditional similar products, featuring advantages such as convenient use, good compatibility, and extremely low background.

UltraBio™ Primary Antibody Dilution Buffer

It is the latest generation of high-efficiency primary antibody diluents. Its overall performance is significantly superior to traditional primary antibody diluents based on BSA (Bovine Serum Albumin), non-fat milk powder, etc. It can be used for the dilution and preparation of primary antibodies in Western Blot (WB) experiments. It has the following advantages:

a. Primary antibody saving: Primary antibodies diluted and prepared with this diluent can be stored and used at 4°C for no less than 6 months, and can be reused multiple times in WB experiments, which is conducive to saving primary antibodies.

b. Extremely low background: This diluent does not contain serum or albumin, ensuring an extremely high signal-to-noise ratio.

c. Grate compatibility: This diluent is compatible with secondary antibodies labeled with Horseradish Peroxidase (HRP), Alkaline Phosphatase, and Biotin in subsequent steps. It does not contain biotin and will not interfere with biotin-based detection.

d. Convenient use: No additional reagents need to be added to this diluent, and it can be directly used for diluting primary antibodies.

Protein-Free Rapid Blocking Buffer

It is the latest generation of fast and high-efficiency Western blocking buffers. Its overall performance is significantly better than traditional blocking buffers based on BSA (Bovine Serum Albumin), non-fat milk powder, Casein, etc., as well as similar foreign products. It is mainly used for blocking PVDF membranes or Nitrocellulose Membranes (NC Membranes) in Western Blot (WB) experiments, and can also be used for diluting primary or secondary antibodies in Western experiments. It has the following advantages:

a. Fast and efficient: When using this blocking buffer, the blocking time usually only takes 5-15 minutes. Compared with traditional Western blocking buffers such as BSA, non-fat milk powder, and casein, as well as similar fast blocking buffers from foreign countries, it exhibits a

stronger signal-to-noise ratio.

b. Extremely low background: This blocking buffer does not contain serum or albumin, ensuring an extremely high signal-to-noise ratio.

c. Grate compatibility: It is compatible with secondary antibodies labeled with Horseradish Peroxidase (HRP), Alkaline Phosphatase, and Biotin. A preservative that does not affect the activity of HRP and AP is added to this blocking buffer, which will not interfere with the detection of HRP or AP-labeled secondary antibodies. At the same time, it does not contain biotin and will not interfere with biotin-based detection.

d. Convenient use: No additional reagents need to be added to this blocking buffer, and it can be directly used for blocking blotting membranes. For the comparison and selection of different blocking buffers, please refer to Aladdin's relevant webpage: http://www.aladdin-e.com/.

ELISA&Western Blot Washing Buffer

It can be used for washing after the incubation of primary or secondary antibodies in Western experiments. Proper washing can reduce the background and enhance the signal-to-noise ratio.

UltraBio™ Protein-Free Secondary Antibody Dilution Buffer for Western Blot

It is the latest generation of high-efficiency secondary antibody diluents. Its overall performance is significantly superior to traditional secondary antibody diluents based on BSA (Bovine Serum Albumin), non-fat milk powder, etc. It can be used for the dilution and preparation of secondary antibodies in Western Blot (WB) experiments. It has the following advantages:

a. Secondary antibody saving: Secondary antibodies diluted with this secondary antibody diluent can be reused 3-5 times within 2-3 weeks.

b. Extremely low background: This diluent does not contain serum or albumin, ensuring an extremely high signal-to-noise ratio.

c. Grate compatibility: It is compatible with secondary antibodies labeled with Horseradish Peroxidase (HRP), Alkaline Phosphatase, and Biotin. A preservative that does not affect the activity of HRP and AP is added to this diluent, which will not interfere with the detection of HRP or AP-labeled secondary antibodies. At the same time, this blocking buffer does not contain biotin and will not interfere with biotin-based detection.

d. Convenient use: No additional reagents need to be added to this diluent, and it can be directly used for diluting secondary antibodies.

Recommended Usage Volume and Kit Capacity

Based on the calculation that each Western Blot experiment requires 10 ml of Blocking Buffer, 10 ml of Primary Antibody Diluent, 10 ml of Secondary Antibody Diluent, and 80 ml of Washing Buffer (10 ml + 30 ml + 30 ml + 10 ml), totaling approximately 100 ml of solutions, one package of this kit can be used for Western Blot detection of approximately 10 membranes.

Components

W751600	Component	4×100ml	Storage
W751600A	UltraBio™ Primary Antibody Dilution Buffer	100ml	2-8℃
W751600B	UltraBio™ Protein-Free Secondary Antibody Dilution Buffer for Western Blot	100ml	2-8℃
W751600C	Protein-Free Rapid Blocking Buffer	100ml	2-8℃
W751600D	ELISA&Western Blot Washing Buffer	100ml	2-8℃

Instructions for Use

1. UltraBio™ Primary Antibody Dilution Buffer

a. Refer to the instructions of the primary antibody used and the content of the target protein in the sample, then dilute the primary antibody at an appropriate ratio (e.g., 1:1000, 1:500). The diluted primary antibody can be directly used for Western Blot (WB) experiments. After one WB experiment, the diluted primary antibody can be recovered and stored at 4°C for use in subsequent WB experiments.

2. Protein-Free Rapid Blocking Buffer

a. After completing membrane transfer, wash the protein membrane with Western Washing Buffer for 1–2 minutes.

b. Depending on the size of the membrane, pour a certain volume of Blocking Buffer into a petri dish or other suitable container, ensuring that the Blocking Buffer can fully cover the membrane afterward. For a regular Western Blot experiment, approximately 10 ml of Blocking Buffer is recommended for a membrane of about 6.6×8.5 cm.

c. Use flat-tipped forceps to hold one corner of the membrane, place the membrane in the Protein-Free Rapid Blocking Buffer (ensuring the membrane is completely immersed), and incubate on a horizontal shaker for about 10 minutes (usually 5–15 minutes is acceptable). Tests with multiple antibodies have shown that a 10-minute blocking time often yields significantly better results than the conventional 1-hour blocking with BSA.

3. ELISA & Western Blot Washing Buffer

a. Take one bottle of ELISA & Western Blot Washing Buffer (10×), pour it into a clean graduated cylinder, add distilled water, mix well, and dilute to a final volume of 1 liter to prepare ELISA & Western Blot Washing Buffer (1×), which can be used for washing in WB experiments. Unused Washing Buffer can be stored at room temperature and is generally usable within 1–2 weeks. If the Washing Buffer becomes turbid, develops precipitates, or shows other abnormalities, it should be discarded.

b. After the incubation of the primary or secondary antibody, add a small amount of ELISA & Western Blot Washing Buffer (1×) to cover the protein membrane. Wash on a shaker for 5 minutes, then aspirate all the Washing Buffer and add fresh Washing Buffer for another wash. Repeat the washing process 3 times, with each wash lasting 5 minutes, before proceeding to subsequent steps. If the background of the WB result is still high under the above washing conditions, the washing time can be appropriately extended and the number of washes increased. Generally, extending the washing time or increasing the number of washes will not

have a negative impact on the WB results.

4. UltraBio™ Protein-Free Secondary Antibody Dilution Buffer for Western Blot

a. Refer to the instructions of the secondary antibody used, as well as the quality of the primary antibody and the content of the target protein in the sample, then dilute the secondary antibody at an appropriate ratio (e.g., 1:1000, 1:500). The diluted secondary antibody can be directly used for WB experiments. After one WB experiment, the diluted secondary antibody can be recovered and stored at 4°C for use in subsequent WB experiments.

b. For detailed Western Blot operation procedures, please refer to our relevant webpage: http://www.aladdin-e.com/support/western.htm

Precautions

1. Use flat-tip tweezers to gently handle PVDF and NC membranes on the corners or edges to avoid producing any scrapes, creases or indentations on the surface of membrane. PVDF membrane, once infiltrated and activated, should be kept wet by placing the membrane in Western transfer buffer or washing buffer according to the Western Blot procedures. Otherwise, an abnormal background that is hard to be blocked may be produced.

2. To enable the diluted antibodies to be reused multiple times, the primary/secondary antibody dilutions should be stored at 4ºC immediately after each use.

3. Usually, it takes 5-15 minutes at room temperature to block PVDF or NC membranes. For some antibodies with very high background, prolong the blocking time to 30-60 minutes at room temperature, or overnight at 4ºC if necessary. Because no blocking reagent is optimal for all experimental systems, a proper blocking buffer should be selected based on the special requirement of your experiment. provides a variety of blocking solutions that you may compare and select.

4. To allow the diluted secondary antibody to be reused multiple times, the diluted secondary antibody should be immediately stored at 4°C after the secondary antibody incubation is completed, facilitating subsequent repeated use.

5. This product is for R&D only. Not for drug, household, or other uses.

6. For your safety and health, please wear a lab coat and disposable gloves during the operation.