RedAlert 10X Western Blot Stain

About the Kit

RedAlert 10X Western Blot Stain	50 ml	71078-3

Description

The RedAlert 10X Western Blot Stain is a concentrated stock solution of RedAlert stain which binds reversibly to proteins on nitrocellulose and PVDF membranes and can also stain proteins bound to cellulose acetate. The RedAlert Stain binds positively charged amino groups and non-polar regions of the protein which results in a red/pink coloring of protein. RedAlert Stain is often used to verify transfer of proteins prior to immunological detection on nitrocellulose or PVDF membranes or to detect blood serum proteins on cellulose acetate media. Since the stain is reversible, it is a good choice for detecting proteins prior to protein sequencing or HPLC analysis. The sensitivity of the stain is approximately 50 ng of protein. The RedAlert Stain is provided as a 10X solution and it is diluted to 1X prior to use.

Components

- 50 ml of RedAlert 10X Western Blot Stain

Storage

The RedAlert 10X Western Blot Stain should be stored at room temperature. Mix or shake the RedAlert 10X Western Blot Stain thoroughly before use.

RedAlert Stain

Staining of proteins bound to nitrocellulose and PVDF membranes

1. Electrophoretically transfer protein samples to nitrocellulose or PVDF membrane.

Note: *Nylon based membranes are not recommended since the stain binds strongly to the membrane.*

2. Wash the membrane in distilled water for 5 min.
3. Place the membrane in 9 ml distilled water and add 1 ml of thoroughly mixed RedAlert 10X Western Blot Stain.
4. Incubate the membrane for 1–5 min (or until desired band intensities are obtained) with gentle rocking at room temperature.
5. Decant and discard the stain.
6. Remove excess RedAlert Stain by gently rocking for 2 min in distilled water.
7. Analyze the protein profile. At this time the protein bands could be photographed, marked with waterproof black ink or cut from the membrane.
8. To remove the RedAlert Stain, wash the membrane in several changes of 0.1 M NaOH in distilled water followed by a distilled water wash for 2–3 min.

Note: *There is no need to completely destain prior to immunological detection. After step 4, place the blot into blocking buffer. The stain will be removed by the blocking buffer.*

Novagen is a brand of CN Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany

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Staining of proteins bound to cellulose acetate

1. After electrophoretic transfer of protein to cellulose acetate, incubate the membrane in sufficient 1X RedAlert Stain to cover the blot for 5 min (or until desired band intensities are obtained) with gentle rocking at room temperature. The RedAlert 10X Western Blot Stain is diluted to 1X with distilled water.
2. Destain in two successive washes in 10% acetic acid (v/v) in distilled water for approximately 5 min at room temperature with gentle rocking until the background stain is removed.
3. Wash the membrane for 5 min in methanol.
4. Wash the membrane for 5 min in clearing solution (Clearing solution consists of methanol:acetic acid:polyethylene glycol [PEG] MW 400 in a ratio by volume 70:30:4.)
5. Dry the membrane.

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