



<u>Catalog No.</u> 5920-0004 (71-83-00) <u>Size</u>

240 mL Solution + 20 g Powder

DESCRIPTION

KPL Detector Block can be used as both a blocking solution and conjugate diluent in membrane-based detection assays. As a blocking solution, it blocks membranes prior to the addition of enzyme conjugates preventing nonspecific binding which would cause background. KPL Detector Block was specially formulated to do this with minimal interaction with specific sites resulting in high levels of sensitivity on membranes. As a diluent for conjugated proteins and antibodies, it minimizes nonspecific binding of the conjugate to other proteins, itself and to the membrane.

CONTENT

5920-0004 (71-83-00) contains: 2 x 120 mL 5X KPL Detector Block Solution 2 x 10 g KPL Detector Block Powder

FORM/STORAGE

5X KPL Detector Block Solution is supplied as a concentrated liquid. The solution should be clear and amber in color. Store solution at 2-8°C. KPL Detector Block Powder is a free flowing, white to light cream-colored powder and should be stored at 2-8°C. Products are stable for a minimum of 1 year from date of receipt when stored under recommended conditions.

APPLICATIONS

KPL Detector Block may be used as a blocking solution for Western blots, Southern blots and Northern blots on a variety of membranes including PVDF, nitrocellulose and nylon. KPL Detector Block may also be used as a diluent for conjugated antibodies and proteins.

USE

Preparation of 1X KPL Block Solution:

- Based on the total desired 1X KPL Detector Block volume, weigh out 0.2% - 1% (w/v) KPL Detector Block Powder depending on application. For detection of alkaline phosphatase, prepare a 0.2% solution; for detection with horseradish peroxidase, prepare a 1% solution. (0.2 - 1.0 grams KPL Detector Block Powder per 100 mL of diluted KPL Detector Block Solution).
- 2. Place the KPL Detector Block Powder in a flatbottom, screw cap container and add molecular

biology grade water to a volume equivalent to 4/5 of the total desired 1X KPL Detector Block volume. Shake the container vigorously until the powder is fully solubilized. (80 mL of H_2O per 100 mL of 1X KPL Block Solution).

 Once the KPL Detector Block Powder is fully solubilized in the water, dilute the solution with 1/5 v/v 5X KPL Detector Block Solution.

Example: for 100 mL of 1X KPL Detector Block for alkaline phosphatase-mediated detection, prepare in the following order:

KPL Detector Block Powder 0.2 g Molecular Biology Grade H₂O 80 mL KPL 5X Detector Block Solution 20 mL

Notes:

- Conical tubes are not recommended in the preparation of 1X KPL Detector Block. If used, the solution may be vortexed to remove any packed KPL Detector Block Powder from the bottom of the tube.
- It is imperative to insure all KPL Detector Block Powder is in solution, otherwise a speckling pattern will appear on the blot or insufficient blocking will occur, resulting in a splotchy black blot.
- The amount of powder used can be increased to reduce background. However, excessive powder may negatively impact sensitivity.
- For maximum sensitivity, it is recommended that the 1X KPL Block Solution be prepared fresh on the day of use.

SUGGESTED PROTOCOLS

The protocols listed below are guidelines for use, it is recommended that the user optimize assay conditions for each particular assay.

Western Blots:

- 1. Following electrophoresis and transfer, immerse the blot in enough 1X Block Solution to allow the membrane to move freely in the solution.
- 2. Incubate at room temperature for 1 hour with agitation or overnight at 4°C without agitation.



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- If blocked overnight at 4°C, equilibrate block and membrane to room temperature for fifteen minutes before proceeding. Dilute antibody or conjugate to the recommended concentration in 1X KPL Block Solution and incubate following standard protocols.
- 4. Detect blot using chemiluminescent or chromogenic substrate following standard protocols.

Note:

 For increased sensitivity on Western blots, the KPL Detector Block Powder may be left out of the 1X KPL Block Solution for the conjugate dilution.

Southern/Northern Blots:

- Following hybridization and post-hybridization stringency washes, block membrane with 1X KPL Block Solution for 30 - 45 minutes at room temperature with gentle agitation.
- Use a minimum of 0.1 0.5 mL 1X KPL Block Solution per cm² of membrane. If using a tray or other large container, add enough 1X KPL Block Solution to completely immerse the membrane.
- Dilute conjugate to the recommended concentration in 1X KPL Block Solution and incubate following standard protocols.
- 4. Use the same volume of solution as was used in the blocking step.
- 5. Detect blot using chemiluminescent or chromogenic substrate following standard protocols.

PRODUCT SAFETY AND HANDLING

See SDS (Safety Data Sheet) for this product.

RELATED PRODUCTS KPL LumiGLO [®] Chemiluminescent Peroxidase Substrate Kit	CAT. NO. 5430-0040 (54-61-00)
KPL Detector™ AP Chemiluminescent Blotting Kit 2000 cm ²	5910-0028 (54-30-01)
KPL Detector™ AP Chemiluminescent Blotting Kit 500 cm ²	5910-0029 (54-30-02)
KPL Detector™ HRP Chemiluminescent Blotting Kit	5910-0027 (54-30-00)
KPL 20X SSC	5960-0021 (50-86-05)
KPL Hybridization Bags	5960-0026 (60-00-51)
KPL Biodyne [®] B Membrane	5960-0025 (60-00-50)
KPL Herring Sperm DNA Kit	5920-0003 (60-00-14)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.