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Anti-Goat Enhanced Alkaline Phosphatase Western Blotting Kit

Product # AP 20 **Typical Lot**

Materials included in the kit:

Donkey anti-Goat Second Antibody	40 µL	(0.6 mg/mL)
Enhancer	200 µL	(4.9 mol/L)
NBT	700 µL	(50 mg/mL)
BCIP	350 µL	(50 mg/mLQ)
*Amido Black	1 mL	

Reagents needed for the assay:

**** DPBS for 1 Liter**

0.20 g KCL
0.20 g KH2PO4
8.00 g NaCl
2.16 g Na2HPO4-7H2O
Adjust the pH to 7.4

**** Tris Buffered Saline** (TBS) pH 7.5 for 500 mL

1.20 g Tris Base (20 mM)
14.6 g NaCl (500 mM)
Adjust the pH to 7.5

**** Tris Buffer** (0.1 M) pH 9.5 for 500 mL

6.05 g Tris Base

Adjust the pH to 9.5

Developing solution

20 mL 0.1 M Tris pH 9.5

20 µl Enhancer concentrate

66 µl NBT ***

33 µl BCIP ***

Amido black solution

1.0 mL of amido black

100 mL of distilled water

Blocking solution (10% milk solution)

10g dry milk

in 100 mL DPBS

Protocol:

1. Remove blot from apparatus.
2. Place it into the Amido black solution
3. As soon as you see bands on the blot take it out and place it in water.
4. Place in blocking solution for at least 2 hours at room temperature.
5. Rinse well with DPBS.
6. Incubate in primary antibody diluted in DPBS for 2 hours at room temperature on a shaker. ****
7. Rinse 3x with DPBS for 10 minutes at room temperature.
8. Dilute secondary antibody 1:5000 in DPBS.
9. Incubate with secondary antibody for 1 hour at room temperature on a shaker.
10. Rinse 3x with DPBS for 10 minutes at room temperature.
11. Rinse 2x with TBS for 10 minutes at room temperature.
12. Incubate in Tris Buffer at room temperature until developing.
13. Place blot in to the Developing solution.
14. Allow reaction to continue until desired band starts showing up. *****
15. Stop color development by placing blot in water.
16. Remove blot from water and dry it on filter paper.

Storage:

The kit should be stored at -20°. Compounds in the amber vials are light sensitive and must be stored accordingly until used.

Notes:

* Reusable and can be stored at room temperature.

** Not provided with the kit but available under separate purchase, if desired. (Product # AP 50, Enhanced Alkaline Phosphatase Western Blotting Kit Buffer System)

*** Make sure NBT and BCIP are warmed to room temperature and are completely dissolved.

**** For primary antibody dilution instructions, refer to manufacturer's specifications.

***** Usually, to develop a blot it takes between 20 seconds to 10 minutes for the desired band to appears. If the blot is kept in the developing solution too long, you might see either non-specific cross reactivity or very strong background. However, if your primary antibody is monoclonal you should be able to keep the blot in the developing solution for a longer period of time.