(dd H_2O). Adjust pH to 9.5 with HCl and bring to 1 liter with dd H_2O.

B. NBT color development stock solution:

Prepare 1 ml of 70% DMF solution by mixing 0.7 ml of DMF with 0.3 ml of dd water. Dissolve 30 mg of NBT in this 70% DMF solution.

C. BCIP color development stock solution:

Dissolve 15 mg of BCIP in 1 ml of DMF.

2. Prepare the color development solution:

Just prior to use, mix 1 ml of the 30 mg/ml NBT stock and 1 ml of the 15 mg/ml BCIP stock into 100 ml of the Tris buffer. Immerse the nitrocellulose membrane in the color develop-ment solution. Protein concentrations greater than 100 ng will become visible immediately. To maximize sensitivity, the incubation can be extended to 4 hours, but this may result in higher backgrounds. If a large amount of precipitate forms before development is complete, decant the color development solution and add additional, freshly prepared, color development solution. The precipitate, which is usually generated by high concentrations of alkaline phosphatase on the membrane surface, will settle on the membrane and can produce unusually high backgrounds.

To stop the reaction, immerse the membrane in dd water for 10 minutes with gentle agitation. Change the water at least once during the 10 minute period to remove residual color development solution. Dry the membrane on filter paper and store between polyester sheets. For further information about the color development assay consult the Immun-Blot assay kit instruction manual.

Reagent Storage	Temperature	Shelf Life
AP Color Development Reagents, BCIP and NBT	-20 °C (desiccated)	1 year
Tris buffer solution (use sodium azide as a bacteriostat)	23-25 °C	1 month
BCIP and NBT stock solution (store in amber vial, protected from exposure to light)	4 °C	3 months

Bio-Rad Laboratories, 2000 Alfred Nobel, Hercules, CA 94547
LIT-324 Rev C

Instructions for Preparing BCIP/NBT Color Development Solution

Catalog Numbers
170-6532 and 170-6539

BIO-RAD

The Alkaline Phosphatase Color Development Reagents, BCIP (5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt) and NBT (p-nitro blue tetrazolium chloride), are used for detecting antigens bound to nitrocellulose or other membranes. These substrates, when used as a pair, develop an insoluble purple product on the membrane surface following exposure to alkaline phosphatase conjugated antibodies.

Reagents Available from Bio-Rad

Catalog Number	Product Description	Quantity
170-6518	**Affinity Purified Goat Anti-Rabbit IgG (H + L) Alkaline Phosphatase Conjugate,** human IgG (H + L) adsorbed, blotting grade	1 ml
170-6520	**Affinity Purified Goat Anti-Mouse IgG (H + L) Alkaline Phosphate Conjugate,** human IgG (H + L) adsorbed, blotting grade	1 ml
170-6521	**Affinity Purified Goat Anti-Human IgG (H + L) Alkaline Phosphatase Conjugate,** bovine IgG adsorbed, blotting grade	1 ml
161-0308	**Biotinylated SDS-PAGE Standards Kit,** Low Range, contains Biotinylated Standards, low range, 250 µl and Avidin-AP,	1 ml
161-0313	**Biotinylated SDS-PAGE Standards Kit**, AP, High Range contains Biotinyl ated Standards, high range, 250 µl and Avidin-AP,	1 ml
170-6432	**Alkaline Phosphatase Conjugate Substrate Kit**, Premixed liquid BCIP and NBT solutions, prepares 1L color development solution	

Catalog Number	Product Description	Quantity
161-0715	**Tris**	100 g
161-0716	**Tris**	500 g

Other Reagents Required to Generate the Color Development Solution

1. Magnesium chloride ($MgCl_2$•$6H_2O$)-ACS reagent grade
2. N,N-Dimethylformamide (DMF)-ACS reagent grade
3. Sodium hydroxide (NaOH)-ACS reagent grade

Procedure to Generate the Color Development Solution

1. Prepare the following solutions.
   A. Tris buffer (0.1 M Tris, 0.5 mM $MgCl_2$, pH 9.5), 1 L:

      Dissolve 12.1 g Tris and 0.12 g $MgCl_2$•$6H_2O$ in 800 ml distilled, deionized water