

DATA SHEET

New Fuchsin Substrate Pack For Use with Alkaline Phosphatase Detection Kits

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REAGENTS SUPPLIED (Store at 2-8°C)

New Fuchsin Substrate Pack (HK183-5K):

- 8 x 5.0 ml of Tris Buffer
- 2.5 ml of New Fuchsin Chromogen Solution
- 2.5 ml of New Fuchsin Activator Solution
- 1 x 5.0 ml of Substrate Solution
- 1 Mixing vial (**NOTE:** thoroughly wash mixing vial in between uses or use a new/clean vial for each use.)

PREPARATION AND USE

Note: New Fuchsin is a permanent substrate for the demonstration of alkaline phosphatase¹. To inhibit endogenous alkaline phosphatase activity, levamisole (HK113-5K) can be added to the Substrate just prior to use at a concentration of 0.6 mg/ml.

1. Remove reagents from storage 15-20 minutes prior to use to allow them to reach room temperature. Prepare fresh substrate immediately prior to use.
2. Combine 50 μ l (1 drop) of New Fuchsin Chromogen Solution and 50 μ l (1 drop) of New Fuchsin Activator Solution in the bottom of the mixing vial. **NOTE:** We recommend that you use a micropipettor to add the correct volume of each of these reagents to the bottom of the vial rather than using the dropper tips supplied. This will help prevent reagents from sticking to the sides of the mixing vial and therefore not mixing properly. **This is a critical step for successful color development.**
3. Mix reagents thoroughly by repeated gentle pipetting.
4. Empty contents of one vial of Tris Buffer (5 ml) into Chromogen-Activator mixture prepared in above steps and mix well.
5. Add 400 μ l (8 drops) of Substrate Solution to the solution prepared in Step 4, and mix well. Substrate is now ready for use. **NOTE:** If endogenous alkaline phosphatase is suspected, add levamisole (HK113-5K) at a concentration of 0.6 mg/ml to the Substrate at this time to inhibit the alkaline phosphatase activity.
6. Add Substrate dropwise to slide, and incubate 15-40 minutes.
7. Counterstain and coverslip. For permanent mounting, mount with BioGenex SuperMount[®] mounting medium (HK079) or equivalent.

REFERENCE

Malkin, N.J., Daymon, M.E. Improved double immunoenzyme labelling using alkaline phosphatase and horseradish peroxidase. J Clin Pathol 35:1092-1094, 1982.