New Fuchsin Substrate System  
Code K0698

Intended use  
For Research Use Only.

The New Fuchsin Substrate System is intended for use as a substrate and chromogen in immunohistochemical staining procedures utilizing alkaline phosphatase. This substrate system is especially useful in immuno-alkaline phosphatase procedures performed on tissue samples.

Summary and principle
New Fuchsin is a commonly used chromogen for alkaline phosphatase. It forms an insoluble red reaction product at the site of the target antigen.

Reagents
Each New Fuchsin Substrate System K0698 contains the following reagents:

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 mL</td>
<td>Tris Buffer Concentrate, pH 8.8</td>
</tr>
<tr>
<td>5 mL</td>
<td>Buffered Substrate Concentrate, pH 8.2</td>
</tr>
<tr>
<td>5 mL</td>
<td>New Fuchsin Solution in 2 N HCl</td>
</tr>
<tr>
<td>5 mL</td>
<td>Activating Agent in distilled water</td>
</tr>
</tbody>
</table>

Precautions
1. For Research Use Only. Not for use in diagnostic procedures.
2. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
3. Unused solution should be disposed of according to local, State and Federal regulations.
4. Safety Data Sheet available for professional users on request.

Warning
Tris Buffer Concentrate: 30-60% Trometamol
H319 Causes serious eye irritation.
H315 Causes skin irritation.
H335 May cause respiratory irritation.
P280 Wear protective gloves. Wear eye or face protection.
P271 Use only outdoors or in a well-ventilated area.
P261 Avoid breathing vapor.
P264 Wash hands thoroughly after handling.
P304 + P340 + IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or physician.
P312 P362-2 + P363 Center or physician if you feel unwell.
P302 + P352 + IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing. Wash contaminated clothing before reuse.
P332 + P313 If skin irritation occurs: Get medical attention.
P305 + P351 + P338 If eye irritation persists: Get medical attention.
P304 + P351 + P338 + P353 + P363 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
P405 Store locked up.
P501 Dispose of contents and container in accordance with all local, regional, national and international regulations.

Danger
New Fuchsin Solution: 5-10% Hydrochloric acid
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves. Wear eye or face protection. Wear protective clothing.
P264 Wash hands thoroughly after handling.
P304 + P340 + IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a POISON CENTER or physician.
P331 + P310 + IF SWALLOWED: Immediately call a POISON CENTER or physician. Rinse mouth. Do NOT induce vomiting.
P330 + P331 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
P338 + P310 Continue rinsing. Immediately call a POISON CENTER or physician.
P405 Store locked up.
P501 Dispose of contents and container in accordance with all local, regional, national and international regulations.
Reagent preparation
Prepare the reagent immediately prior to application.

**Substrate:**
1. Place 2 drops of the Tris Buffer Concentrate into a test tube.
2. Bring up volume to 2 mL with distilled water.
3. Add 1 drop of the Buffered Substrate Concentrate and mix contents gently.

**Chromogen:**
1. Place 1 drop of the New Fuchsin Solution and 1 drop of the Activating Agent into an empty test tube. Mix gently.
2. Allow to stand for 3 minutes.

**Substrate-Chromogen Reagent:**
1. Transfer the substrate reagent into the test tube containing the chromogen.
2. Mix.

**Storage**
All reagents should be stored in the dark at 2–8 °C.

**Materials supplied**
New Fuchsin Substrate System contains sufficient reagents for staining a minimum of 80 tissue specimens or 40 cell smears.

**Materials required, but not supplied**
2 glass test tubes
Pipette to deliver 2 mL
Transfer pipettes

**Procedure**
Following incubation with an alkaline phosphatase-labelled reagent, place specimens in buffer bath. Tap off excess buffer and carefully wipe slide around specimen.

1. Cover specimen with the prepared Substrate-Chromogen Reagent. Incubate 5–10 minutes. Optimal incubation time may vary with antigen and/or specimen fixation and should therefore be determined by the individual laboratory.
2. Counterstain, if desired.
3. Coverslip with mounting media.

**Results**
Use of the New Fuchsin chromogen yields a red reaction product at the site of the target antigen. Diffuse pink staining, if any, of connective tissue is likely nonspecific and should be disregarded.

For proper interpretation, positive and negative controls should be run alongside unknown specimens. Positive controls serve as indicators that specimen processing and handling were carried out correctly. Negative controls are useful for assessing nonspecific staining.

**Limitations**
Endogenous alkaline phosphatase activity in most human cells, with the principal exceptions of intestinal epithelial and placental syncytiotrophoblast cells, can be inhibited by the use of levamisole (code X3021) in the substrate solution (1). Levamisole does not inhibit endogenous alkaline phosphatase found in small intestine and stomach (1).

**References**
1. Ponder BA, Winlinson MM. Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. J Histochem Cytochem 1981;29(8):981-4