Technical Data Sheet

Technovit H7100 / H8100 Acid Phosphatase

14653-14654

Acid Phosphatase For Glycol Methacrylate Sections

Procedure

1. Incubate sections in the incubating medium at 37°C for five to 12 hours. Long incubation periods are needed to get significantly visible reaction product.
2. Wash in distilled water for two minutes.
3. Counterstain with Methyl Green for five minutes.
4. Wash in distilled water for two minutes.
5. Air dry and cover slip.

Results

Nuclei	dark green
Cytoplasm	light green
Sites of enzyme activity	red

Solutions

Incubating Medium:

- Combine 20ml of buffer solution, 48ml of distilled water and 4ml of substrate solution.
- Combine 3.2ml of Pararosaniline solution with 3.2ml of sodium nitrite solution. Mix for one minute.
- Add the second solution to the first.
- Adjust pH to 5.

Buffer Solution:

- 5.9 g Anhydrous sodium acetate
- 14.7g Sodium barbiturate
- 500ml Distilled water (boiled)

Do not adjust the pH of the buffer and store at 4°C.

Substrate solution:

- 40mg Naphtol As-BI phosphatease, sodium salt
- 4ml N.N-dimethylformamide

Pararosaniline Solution:

- 2g Pararosaniline (C.I.#42500)
- 50ml 2N HCl

Use heat to dissolve, filter when cool and store at 4°C

Sodium Nitrite Solution:

- Sodium Nitrite - 1 gm
- Distilled Water - 25 ml

Prepare fresh and store at 4°C.

Methyl Green

- Methyl Green (C.I.# 42585) - 1 g
- Phosphate/citrate buffer 0.1M pH 4.0 - 100 ml

Citation:

Gerrits, P. O. and Smid, L., "Staining Procedures for Tissues Embedded in 2-Hydroxyethyl Methacrylate", Heraeus Kulzer.