BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Mast Cell Staining Kit (Cat #: A-127)

COMPONENTS: Toluidine Blue: 20 ml, store at room temperature
Toluidine Blue Buffer: 200 ml, store at room temperature

PRODUCT DESCRIPTION: Mast cells are found in the connective tissue and best known for their roles in mediating inflammatory responses such as hypersensitivity, allergy and anaphylaxis. Mast cells prominently store potent chemical mediators such as histamine, interleukins, proteoglycans and various enzymes in secretory granules. In a process referred to as degranulation, mast cells release the contents of the granules into the surrounding tissue, triggering local inflammatory reactions exemplified by mucus production, increased permeability of blood vessels and contraction of smooth muscle. The mast cell staining method is based on an optimized formulation of Toluidine blue, a basic thiazine metachromatic dye with high affinity for acidic tissue components. Toluidine blue has found wide applications both as vital staining in living tissues and as a special stain to highlight mast cell granules, mucins and cartilage. The staining solution should stain mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining) in a deparaffinized section. Assay reagents are stable for several years if stored and handled properly.

PROTOCOL:

Preparation of working staining solution

To prepare working staining solution, mix 1 part of Toluidine Blue with 9 parts of Toluidine Blue Buffer. Mix solution well to use. Prepare solution fresh before use and discard solution after each experiment.

Preparation of fixation solution (not included in the kit)

For best result, prepare a 4% paraformaldehyde solution prior to staining. In the fume hood, add 4 g of paraformaldehyde powder to 100 ml of normal phosphate-buffered saline (PBS). Stir solution with heat on (60-80°C) to dissolve powder. This preparation may take up to one hour for complete dissolution of the paraformaldehyde powder. Cool down the fixation solution to room temperature prior to use.

Cell fixation (for monolayer cells)

Aspirate off cell culture medium. Rinse cells once with PBS and remove PBS. Immerse cells in the freshly prepared paraformaldehyde solution for 5 min. Aspirate off paraformaldehyde completely, and wash cells three times (3 min each wash) with PBS. Aspirate off PBS completely after each wash.

Staining (for fixed cells and deparaffinized slides)

Immerse fixed cells and deparaffinized tissue slides in enough freshly prepared working staining solution. Incubate for 5 min or until blue cells become visible under microscope. Wash samples with dH_2O several times. Cells and tissue slides are now ready for imaging.

Additional information:

- Paraformaldehyde is extremely toxic, and should be handled in the fume hood with caution. The staining solution contains acetic acid, hydrochloric acid, ethanol and Toluidine Blue. Please visit the product webpage or contact us for MSDS information.
- For preparation of 20x PBS, please visit http://www.bmrservice.com/TechNotes.html. Dilute 20x PBS 20-fold with dH_2O to obtain 1x PBS for use.