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

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Chondrogenesis Assay Kit (Cat #: A-105)

COMPONENTS: Alcian Blue Solution- 100 ml, store at room temperature

PRODUCT DESCRIPTION: Alcian blue, a group of polyvalent basic dyes that are water soluble, stains acid mucosubstances (sulfated and carboxylated mucopolysaccharides) and sialomucins (glycoproteins), both of which are cartilaginous elements. Alcian blue is one of the most widely used cationic dyes for the demonstration of glycosaminoglycans. It is thought to work by forming reversible electrostatic bonds between the cationic dye and the negative (anionic) sites on the polysaccharide. Since the synthesis of these extracellular macromolecules is increased during chondrogensis, staining with Alcian blue is an easy method to identify tissues and cells undergoing chondrogenesis. Cell-bound Alcian blue can be extracted after staining and washing, allowing quantification of chondrogenesis by measuring optical density at 605 − 615 nm. Normal phosphate-buffered saline (PBS), 4% paraformaldehyde and 1N HCl are not provided.

PROTOCOL:

Cultured cells-

The protocol is optimized for cells grown on 35-mm culture dishes. However, it can be modified and scaled up for culture vessels of different size.

1. Aspirate off culture medium and wash cells twice with PBS. Remove PBS solution completely. Please visit http://www.bmrservice.com/TechNotes.html for preparation of 20x PBS.
2. To each 35-mm dish, add 0.5 ml 4% paraformaldehyde. Fix cells for 5 min at room temperature. Please visit http://www.bmrservice.com/TechNotes.html for preparation of 4% paraformaldehyde.
3. Aspirate off paraformaldehyde completely and rinse fixed cells twice with distilled water. Remove water completely.
4. To each 35-mm dish, add 0.5 − 1 ml Alcian Blue Solution. Gently agitate dish at room temperature for 30 min.
5. Aspirate off Alcian Blue Solution completely. Wash stained cells with distilled water for 30 sec. Remove water and repeat washing twice. Stained cells can be imaged at this point. For quantitative analysis, proceed through steps 6 − 7.
6. Remove water from dish and add 1 ml 1N HCl to each dish to extract cell-bound Alcian Blue. Agitate dish for 30 min to ensure complete dye extraction. Harvest the HCl solution.
7. Measure O.D. at 615 − 620 nm using 1N HCl as blank. The amount of Alcian Blue bound to cells is directly proportional to $O.D._{615 - 620\ nm}$.

Tissues-

Alcian Blue Solution can also be used to stain deparaffinized tissue sections. Immerse deparaffinized tissue section in Alcian Blue Solution for 30 min followed by gentle rinsing with distilled water. The section can be counterstained in nuclear fast red solution for 5 min (optional) followed by rinsing with distilled water prior to imaging.

NOTE:

- 4% paraformaldehyde should be prepared in a fume hood. The fixative is highly toxic. Avoid inhalation and skin contact.
- Alcian Blue Solution contains acetic acid. Avoid skin contact.
- Please contact us or visit the product webpage for MSDS information on paraformaldehyde, hydrochloric acid, acetic acid and Alcian Blue.