

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Chondrogenesis Assay Kit (Cat #: A-105)

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

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 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 chondrogenesis, Alcian blue staining 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. The Alcian Blue Solution is good for many years if handled and stored properly.

PROTOCOL:

Cell staining:

The protocol is optimized for monolayer cells grown on 35-mm culture dishes. However, it can be modified for various cell culture settings.

1. To each 35-mm dish containing PFA-fixed cells, add 0.5 ml Alcian Blue Solution. Gently agitate dish at room temperature for 30 min. Check cells under an inverted microscope to make sure dark blue stains are visible. The incubation time can also be increased.
2. Aspirate off solution completely and rinse cells three times with distilled water. Remove water completely. Cells are ready for imaging.
3. For quantitative analysis, remove water from dish and add 0.5 ml 1N HCl to each dish to extract cell-bound Alcian Blue. Agitate dish for 30 min to ensure complete dye extraction. Harvest HCl solution from each dish.
4. Measure O.D. at 615 – 620 nm using 1N HCl as blank. The amount of Alcian Blue bound to cells is proportional to O.D..

Tissue section staining:

1. Use paraffin-embedded tissue sections for the staining. Deparaffinize tissue sections first.
2. Immerse slides in Alcian Blue Solution for ~30 min followed by rinsing with distilled water three times. The tissue sections are ready for imaging.

NOTE:

- Paraformaldehyde (PFA) and 1N HCl are not provided with the kit.
- PFA 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 SDS information on PFA, HCl, acetic acid and Alcian Blue.