

Detection of glycosaminoglycans and fibrous collagen in marine fish cell lines

PREAMBULE. Volumes and quantities given below are for a well of a 24-well plate.

GLYCOSAMINOGLYCAN DETECTION

1. Discard medium and wash cell culture 3 times with 1 ml PBS.
2. Fix cells for 1 h at 4°C with 0.5 ml of 4% (v/v) formalin (in PBS).
3. Discard fixative and wash cell culture 3 times with 0.5 ml of 0.1 N HCl.
4. Stain glycosaminoglycans for 5 h at room temperature with 0.5 ml of 0.5% (w/v) alcian blue 8GX solution (in 0.1 N HCl).
5. Discard stain and wash cell culture extensively with MilliQ water.
6. Observe coloration under an inverted microscope and document with micrographs and/or a plate scan.
7. Discard water and extract dye in 100 µl of 1% (w/v) SDS using a cell scraper.
8. Collect extract, pellet cell debris for 5 min at 10000 rcf then measure absorbance of the supernatant at 350 nm (dilute if necessary).

FIBROUS COLLAGEN DETECTION (adapted from Tullberg-Reinert & Jundt, 1999)

1. Discard medium and wash cell culture 3 times with 1 ml PBS.
2. Fix cells for 1 h at room temperature with 0.5 ml of Bouin's fluid.
3. Discard fixative and wash cell culture with running tap water for 15 min. Discard remaining water.
4. Stain collagen fibres for 1 h at room temperature with 0.5 ml of 0.1% (w/v) sirius red F3BA solution (in saturated picric acid solution).
5. Discard stain and wash cell culture extensively with 0.01 N HCl.
6. Observe coloration under an inverted microscope and document with micrographs and/or a plate scan.
7. Discard HCl and destain for 15 min at room temperature with 200 µl of 0.1 N NaOH solution.
8. Collect extracts, pellet cell debris for 5 min at 10000 rcf then measure absorbance of the supernatant at 550 nm (dilute if necessary).

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Apparatus: An inverted microscope equipped with phase contrast.

Solutions: phosphate-buffered saline solution (PBS: 137 mM NaCl, 2.7 mM KCl, 15.8 mM Na₂HPO₄, 1.23 mM KH₂PO₄; adjust pH to 7.4); Bouin's fluid: 71% saturated picric acid solution, 8.5% formalin and 4.8% glacial acetic acid; Sirius red solution: 0.1% sirius red F3BA dye (Chroma-Waldeck) prepared in a saturated picric acid solution.

Plasticware: 24-well cell culture dishes and serologic pipettes.

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Tullberg-Reinert H, Jundt G (1999) In situ measurement of collagen synthesis by human bone cells with a Sirius Red-based colorimetric microassay: effects of transforming growth factor β 2 and ascorbic acid 2-phosphate. *Histochem Cell Biol* 112:271-276