Blyscan GAG Isolation and Concentration Pack (Cat. No. B1015)

(Contains 1 x 100 mL Buffered cetylpyridinium chloride and 2 x 20 mL 2M Lithium chloride).

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Recommended pretreatment of amniotic fluid glycosaminoglycans:

Isolation of glycosaminoglycans from amniotic fluid is recommended prior to attempting GAG measurement.

A modification of the method of Hopwood & Harrison (1982) can be applied to the precipitation of glycosaminoglycans from amniotic fluid. Consistent and near-quantitative recoveries of glycosaminoglycans (~95%) can be achieved using this method.

The pretreatment protocol also provides a convenient method to concentrate the glycosaminoglycans from samples, thus permitting the measurement of very low glycosaminoglycan levels.

- Amniotic fluid samples should always be clarified by centrifugation at >10,000 rpm for 10 minutes.
- An aliquot of this supernatant (0.25 mL) is then mixed with an equal volume of 0.2M sodium citrate buffer, pH 4.8, containing 0.2% (w/v) cetylpyridinium chloride (CPC).
- The above mixture is then incubated at 37°C for 2 hours before centrifugation at >10,000 rpm for 10 minutes.
- The supernatant is decanted and the centrifuge tube, containing the pellet, is drained by inversion on to layers of absorbent paper. The pellet is mixed with 1000 µL of ethanol and held at 37ºC for 5 minutes; this step removes excess detergent into solution.
- Following centrifugation, at >10,000 rpm for 10 minutes, the supernatant is again decanted and the tube, containing the pellet, drained by inversion on layers of absorbent paper.
- After briefly drying the pellet at room temperature, Blyscan dye reagent (1 mL) is added directly to the precipitate in the micro-centrifuge tube and the assay continued according to the standard Blyscan procedure.

Recommended pretreatment protocol for urinary glycosaminoglycans:

Due to the variations that can occur in urinary volumes and, therefore, in the concentration levels of the many components that are present in urine, it is recommended that glycosaminoglycans should be isolated from possible interference using the following 'cleanup' protocol.

This protocol can also provide a convenient method to recover the glycosaminoglycans in a smaller volume, thus permitting the measurement of lower glycosaminoglycan levels.

A modification of a method for the precipitation of glycosaminoglycans from urine by Hopwood & Harrison (1982) has been found to give consistent and near-quantitative recoveries of glycosaminoglycans

- Urine samples (freshly collected) should first be clarified by centrifugation at 10,000g, for 10 minutes at 25°C.
- An aliquot of the transparent urine supernatant (0.75 ml) is mixed with an equal volume of 0.2 M sodium citrate buffer, pH 4.8, containing 0.2% (w/v) cetylpyridinium chloride (CPC).
- The mixture is incubated at 37°C for 2 hours before centrifuging at 10,000g for 10 minutes.
- The supernatant is decanted and the centrifuge tube, with the GAG pellet, drained by inversion on layers of absorbent paper.
- The pellet is dissolved in 2 M LiCl (150 µl) before mixing with 800 µl of ethanol, then held at 37°C for 5 minutes and centrifuged again.
- Following centrifugation, at 10,000g for 10 minutes, the supernatant is decanted and the tube/pellet drained by inversion on layers of tissue paper.
- The pellet is re-suspended in a small volume of de-ionised water (250 µl). Aliquots of this solution are used for the assay.