

# Tissue Digestion Kit

Catalog #K-8001

## DESCRIPTION

Proteoglycans are an integral part of the extracellular matrix for most tissues with the highest amounts found in articular cartilage. They are composed of a protein core with glycosaminoglycans attached. The sulfated glycosaminoglycans can be measured using the Astarte Biologics Proteoglycan Detection kit after treatment with papain which will liberate the glycosaminoglycans from the proteins. This kit is designed to digest small tissue samples, serum, plasma and other biological fluid to enable measurement of the glycosaminoglycans by removing the protein portion of proteoglycans.

## KIT COMPONENTS

- ▶ Lyophilized Papain (3.3 mg/vial)
- ▶ 10x Assay Buffer (10 mL)
- ▶ 1M Tris-HCl pH 8 (1 mL)
- ▶ 1M Acetic acid (1 mL)

## REAGENTS REQUIRED (NOT SUPPLIED)

- ▶ 100 mM Dithiothreitol (DTT) in water. (This reagent can be used freshly prepared on ice, or in advance and stored in aliquots at -20° C.)
- ▶ distilled water

## EQUIPMENT

- ▶ Heat block or water bath at 60° C
- ▶ Microcentrifuge
- ▶ Microcentrifuge tubes (1.5 ml size)

## PROCEDURE FOR TISSUES AND CELLS

1. Prepare tissue samples by mincing tissue with a scalpel and placing into a sterile 1.5 ml microcentrifuge tube. Samples should be 2-10 mg wet tissue weight and weight should be recorded to allow later calculation of the micrograms of proteoglycan per mg. If digesting serum, use 100  $\mu$ L of serum.
2. Reconstitute papain using 1.1mL 10x assay buffer.
3. Add 100  $\mu$ L of reconstituted papain to each tissue sample. Add DTT to a final concentration of 2mM (20  $\mu$ L of 100 mM DTT) and add water up to 1mL.
4. Vortex and incubate at 60°C for at least one hour. If solid soft tissue remains, vortex and incubate until tissue is completely liquified.
5. Add 10  $\mu$ L Acetic Acid and mix well.
6. Add 40  $\mu$ L Tris-HCl (pH 8.0) and mix well. The sample is now ready to proceed to Proteoglycan assay using the **Astarte Proteoglycan Detection Kit (Cat # K-8000)**.

## EXPECTED RESULTS/SAMPLE DILUTION TIPS

1. Dilute all samples at least 1:10 with 1x Assay Buffer before using in proteoglycan assay. Samples diluted less than 1:10 may be affected by papain inhibition.
2. Dilute cartilage samples a minimum of 1:100. Proteoglycans can make up 15-40% of the dry weight found in articular cartilage.
3. Muscle, fat, and serum samples tend to work well at a 1:10 dilution.
4. Test samples at a few different dilutions if uncertain of the proteoglycan content in your sample.
5. Muscle, fat, and serum should have between 10-100  $\mu$ g/mL proteoglycans when prepared using this kit.
6. Cartilage should have between 100-1000  $\mu$ g/mL proteoglycans.