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NB600-504 Protocol

Protocol specific for Aggrecan Neoepitope Antibody (NB600-504)

This protocol is from the reference Roberts S et al. Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. Spine 25:3005-13 (2000). PubMed: 11145811

1. Immunostaining with enzyme-generated antibodies BC3, 13, 4, and 14 was carried out on 19 discs from patients 11-59 years of age (1 with prolapse, 2 with scoliosis, 2 with spondylolisthesis, and the remainder with degenerative disc disease).

2. Different fixation treatments were used to optimize antigen preservation and staining of the cryosections; for example, 70% ethanol, 100% ethanol, 10% formaldehyde, or no fixation was used.

3. After this treatment, sections to be stained for BC 3 and 14 were digested with keratanase I, II, and chondroitinase ABC for 3 hours, and those to be stained for BC4 and 13 were digested with chondroitinase ABC for only 90 minutes.

4. Labeling was as for paraffin sections with other antibodies.

5. Staining was done on 5 um paraffin sections that then were deparaffinized with xylene and rehydrated through a series of alcohols to phosphate buffered saline (PBS). Staining conditions were optimized for each individual antibody... no pretreatment was found necessary.

6. Sequential blocking of endogenous peroxidase activity was performed with 0.3% hydrogen peroxide in PBS and then 20% normal human and horse serum and 3% bovine serum albumin in PBS.

7. The primary antibody was incubated for 90 minutes at room temperature before labeling with peroxidase linked to a biotin-streptavidin complex using diaminobenzadine as the colorimetric substrate, then washing, dehydrating, and mounting in pertex.

Adjacent control sections were incubated with either a class-matched immunoglobulin raised to an irrelevant antigen or PBS in place of the primary antigen.