PROTOCOLS FOR BIOTIN-HABP (BOVINE NASAL CARTILAGE)

Immunohistochemistry for Hyaluronic acid using B-HABP

Reagents

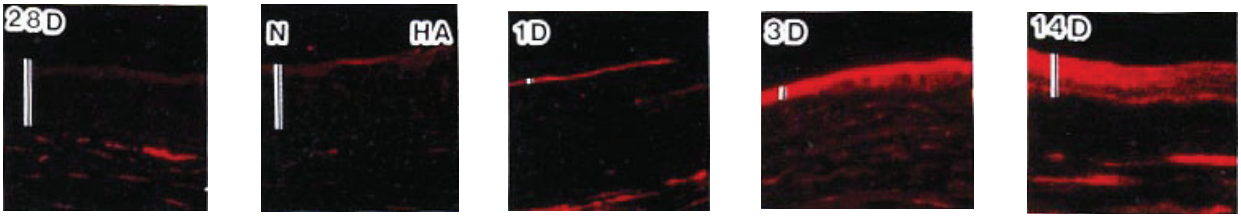
Product	Source	Cat.No.
Biotinylated Hyaluronic Acid Binding Protein	**Seikagaku**	**400763**
Hyaluronidase (*Streptmyces hyalurolyticus*)	**Seikagaku**	**100740-1**
Chondroitinase ABC Protease Free (*Proteus vulgaris*) *	**Seikagaku**	**100332-1A**
Trypsin *	Sigma	
Avidin solution/ Biotin solution	Vector	
Fluorophore conjugated Streptavidin	Jackson	

** For proteoglycan digestion when HA is masked.*

Experimental procedure

1. Pretreatment with Hyaluronidase (Negative control)
Treat with reaction buffer of Hyaluronidase (100mM Sodium acetate buffer, pH6.0) for 15min at 37℃.
No wash
Treat with Hyaluronidase (200TRU/mL; 100mM Sodium acetate buffer, pH6.0) for 2hrs at 60℃.
Wash with PBS
2. Blocking endogeneous avidin biotin activity
Treat with avidin solution for 20min at RT.
Wash
Treat with biotin solution for 20min at RT.
Wash
3. Treat with 0.1% BSA solution for 1hr at RT.
Wash
4. Treat with Biotinylated HABP (2µg/mL) for 1-2hrs at RT.
Wash
5. Treat with Fluorophore conjugated Streptavidin for 15min at RT.

Example: Histochemical staining of hyaluronan (Texas red) in rabbit cornea.
N: Normal cornea; **1, 3, 14** and **28 days**: corneas 1, 3, 14 and 28 days after wounding



ELISA Procedure for Hyaluronic Acid using B-HABP

1. Coat Costar plate with HA (1 ug) or use BSA-HA plates
2. Wash with T-PBS buffer (200ul) 3 times
3. Add bHABP (e.g. 50 ng/mL) [T-PBS/1% BSA as a negative control]
4. Incubate covered by Parafilm at 37 C for 1 hour
5. Discard excess bHABP
6. Wash with T-PBS buffer (200ul) 3 times
7. Add 100ul HRP-sAv solution (ImmunoPure® Streptavidin, Horseradish Peroxidase Conjugated, 1mg)
8. Incubate covered by Parafilm at 37 C for 1 hour
9. Discard excess HRP-SAV
10. Wash with T-PBS buffer (200ul) 3 times
11. Add 100 ul TMB substrate
12. Incubate covered by Parafilm at 37 C for 15 min (prevent light exposure)
13. Add 100ul 1N HCl
14. Measure the endpoint at A450 minus A630nm

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