FITC-Bovine Type II Collagen

Catalog # 4010

For Research Use Only - Not Human or Therapeutic Use

DESCRIPTION: FITC-labeled bovine type II collagen (1, 2)

APPLICATION: Prepare scaffolds in three-dimensional (3D) gels to assay collagenase activity against type II collagen in cultured cells

QUANTITY: 10 mg

FORM: Lyophilized powder

SOURCE: Bovine articular cartilage

MOLECULAR WEIGHT: 300 kDa (molar ratio of collagen to FITC is approximately 1:1)

PURITY: >95% by SDS-PAGE

NOTE: The background fluorescence intensity (FI) value should be less than 0.1% of the control values (caused by free FI extracted with 35% ethanol at 25°C)

STORAGE: Store at 4°C in the dark

STABILITY: 1 year after the shipping date

PROTOCOL:

A. THREE-DIMENSIONAL GEL PREPARATION PROTOCOL

NOTE: Gels can be prepared just prior use

1. Dissolve 1 vial of FITC-collagen in 2.5 ml of 0.01M acetic acid (final concentration: 4 mg/ml)

2. Prepare a gel solution using the following table

Reagent	Volume
FITC-bovine type II collagen dissolved in 0.01M acetic acid (4 mg/ml)	2.5 ml
Cat # 1202 Cell Culture Grade Bovine Type I Collagen (4 mg/ml x 12.5 ml) **OR** Cat # 1062 Immunization Grade Bovine Type I collagen (4 mg/ml dissolved in 0.01M acetic acid)	2.5 ml
1M Phosphate Buffer pH 7.4 containing 1.5M NaCl	0.67 ml
Cold Distilled Water	1 ml
0.5% Phenol Red Solution	20 µl

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3. Adjust the pH to 7-8 using 6 N NaOH until the solution becomes light red in color (check the pH using pH test strips)

4. Aliquot the desired volume (e.g. 300 µl/well in a 24-well plate).

Optional: Cells can be suspended directly in the collagen solution for embedding cells in the entire scaffold (*3*).

5. Incubate at 37°C for 30-60 minutes.

6. The gel is now ready to use

B. CELL-BASED COLLAGENASE ACTIVITY ASSAY(*3*)

1. Seed the cells onto the scaffold (or use cell-embedded collagen gels).

2. Culture the cells as needed.

3. Measure FI values of the culture medium.

NOTE 1: Culture medium typically contains phenol red, which can cause quenching and affect the final FI values. Chondrex, Inc. recommends using the same medium for the blank and 100% values in addition to the samples.

NOTE 2: The 100% value can be obtained by completely digesting the cell-free collagen scaffold with bacterial collagenase (Cat # 30141). The degradation rate of type II collagen can be calculated using the FI value of the samples. Please contact order@chondrex.com to order Cat # 30141.

NOTES:

The combination of type I and type II collagen creates biomimetic heterotypic fibrils that provide the high structural stability of type I collagen combined with the native biochemical signaling of type II collagen. This method provides accurate analysis of cell-specific collagenase cleavage kinetics (*4*).

REFERENCES:

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