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Technical Datasheet

EZStain™ Chondrocyte Staining Kit

Product Code: CCK029

1. Introduction

Chondrocytes are mature cells found in cartilage. They make up the cellular matrix of cartilage. The matrix consists of aggrecan and collagen. Aggrecan is the major proteoglycan present in the cartilage. CCK029, EZStain™ Chondrocyte Staining Kit is a ready-to-use kit developed for detection of chondrocytes by staining the proteoglycan produced by them.

2. About the Kit

Alcian blue is a basic dye that stains acidic proteoglycan present in cartilage. It specifically binds sulfated glycosaminoglycan chains present in the chondrocyte matrix and stains them blue in colour.

3. Applications

- Identification of chondrocytes in tissue
- Alcian blue is used in clinical histology for diagnosis of gastro-intestinal tract diseases
- Detection of connective and epithelial tissue tumor
- Detection of source of collagen diseases and atherosclerosis

4. Kit contents

Code	Contents	Quantity
CCK029(A)	Washing Solution	1 X 20ml
CCK029(B)	Fixing Solution	1 X 20ml
CCK029(C)	Staining solution	1 X20ml

Note: Store all the reagents at room temperature

5. Materials required but not provided in the kit

- Chondrocytes / Cartilage tissue
- Sterile water
- 0.1N Hydrochloric acid
- Microscope with 40X or higher objectives
- Microscopy slides
- Cover slips
- Scalpel
- Multi-well plates
- Serological pipettes

6. Directions for use

Users are advised to review entire procedure before starting the assay

6.1 Procedure for staining cultured cells in plates

6.1.1 Washing the cells

1. Transfer the chondrocytic spheroids formed as a result of chondrocytic differentiation from three-dimensional culture system to cell culture multi-well plate.
2. Aspirate off the spent medium from all the wells.
3. Add sufficient volume of washing solution along the side of each well to cover spheroids completely.
4. Swirl gently to wash the spheroids.

6.1.2 Fixation of cells

1. Aspirate off the washing solution and add sufficient volume of fixing solution to each well to cover the spheroids completely.
2. Incubate the plate at room temperature for 30 minutes in fume hood.
3. After incubation aspirate off the fixing solution and add sufficient volume of distilled water along the side of each well to cover spheroids completely.
4. Swirl gently to remove any traces of fixing solution.

6.1.3 Staining the cells

1. Aspirate off the fixing solution and add sufficient volume of staining solution
2. Incubate the plate at room temperature for 30 minutes.
3. After incubation aspirate off the staining solution and rinse with 0.1N HCl for 2 - 3 times.
4. Add distilled water to neutralize the acidity.
5. Observe the proteoglycan stained with blue colour under light microscope or phase contrast microscope.

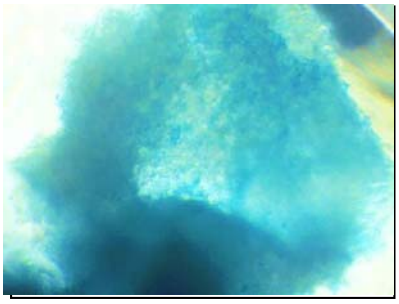
6.2 Procedure for staining tissue

1. Place a very thin piece of tissue on a clean,grease-free microscopy slide.
2. Mince it with the help of scalpel.
3. Very small unminced pieces of tissue can be left on the slide.
4. Place the slide on a sheet of tissue paper.
5. Put staining solution on the tissue in a quantity sufficient to cover the tissue.
6. Place long coverslip on the tissue across the slide.

   *(**Note:** Avoid trapping of bubbles while placing the coverslip in tissue. Presence of bubbles may interfere with microscopic observation.)*
7. Press the coverslip uniformly across the length of the slide to squash the tissue between coverslip and slide.
8. Incubate at room temperature for 30 minutes.
9. Fix the coverslip on slide with the help of transparent nail polish.
10. Observe under phase contrast microscope at 40X magnification.

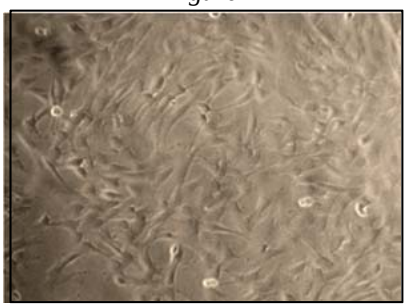
7. Observations

Figure 1



Proteoglycan in mesenchymal stem cells differentiated into chondrocytes stained with Alcian blue

Figure 2



Undifferentiated Human Adult Mesenchymal Stem cells (40X)

8. Storage and Shelf Life

- Store all the reagents at room temperature.
- If precipitation occurs in staining solution, filter it through Whatmann filter paper before use. Precipitation and subsequent filtration does not affect performance of the staining solution.
- Use contents before expiry date given on the label.

9. Related products:

HiDiff™ 3T3-L1 Differentiation kit
Code: CCK011-100NO

Xpert™ 3T3-L1 Differentiation Teaching Kit
Code: CCK021-25NO

EZstain™ Adipocyte Staining Kit
Code: CCK013-1KT

EZstain™ Osteocyte Staining Kit
Code: CCK030-1KT

EZstain™ Hepatocyte Staining Kit
Code: CCK014-1KT

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

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