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Modified Safranin O–Fast Green Cartilage Staining Kit

1 Packing list

Components	HY-K0607-250 mL	HY-K0607-500 mL
Weigert Solution A	25 mL	50 mL
Weigert Solution B	25 mL	50 mL
Acidic Ethanol Differentiation Solution	50 mL	100 mL
Fast Green Staining Solution	50 mL	100 mL
Acetic Acid Solution	50 mL	100 mL
Safranin O Staining Solution	50 mL	100 mL

2 Introduction

Cartilage is a specialized connective tissue characterized by the absence of blood vessels, nerves, and lymphatic vessels. It is mainly composed of chondrocytes, extracellular matrix, and fibrous components. Cartilage is widely distributed in synovial joints, the vertebral column, ribs, external ear, nose, and airways, as well as in the growth plates of children and adolescents. Based on differences in tissue structure and matrix composition, human cartilage can be classified into three major types: hyaline cartilage, fibrocartilage, and elastic cartilage. In histological research and pathological analysis, specific staining methods are commonly used to observe and differentiate cartilage tissues. Frequently used methods include toluidine blue staining, Alcian blue staining, and Safranin O staining.

Safranin O is a cationic dye that binds to negatively charged groups in cartilage matrix proteoglycans, such as chondroitin sulfate and keratan sulfate, resulting in red, reddish-purple, or purple staining of cartilage. The staining intensity of Safranin O generally correlates with the amount of anionic groups present in the tissue, and therefore can reflect, to some extent, the content and distribution of proteoglycans in the cartilage matrix. For example, when cartilage tissue undergoes injury or degeneration, proteoglycans within the matrix may be degraded or lost, leading to reduced staining intensity or uneven staining patterns.

Fast Green is an acidic dye that binds to acidophilic components in tissues, staining bone tissue or collagenous matrix green or bluish-green. When used in combination with Safranin O, it allows effective differentiation between cartilage and bone within the same tissue section, thereby enhancing the contrast of tissue structures.

MCE Modified Safranin O–Fast Green Cartilage Staining Kit is developed based on the classical staining principles described above. Through an optimized staining system, cartilage matrix and bone tissue in histological sections can be clearly distinguished. This method provides high staining contrast, good sensitivity, simple operation, and good reproducibility, and is widely used for cartilage morphology observation, studies of cartilage injury and degeneration, osteochondral tissue development research, and related histological analyses.

This product is suitable for staining experiments using paraffin-embedded tissue sections.

3 General Protocol

Reagents Preparation

- Fixative: 10% Formalin
- Other reagents: Decalcification solution, Xylene, neutral mounting medium or mounting reagent, graded ethanol solutions, distilled water, etc.

Procedure

1. Sample Preparation
   1) After fixation in 10% neutral buffered formalin, tissue samples should undergo decalcification, routine dehydration, and paraffin embedding to prepare paraffin sections.
   2) Sections should be deparaffinized in xylene or a deparaffinization/clearing reagent, followed by rehydration through graded ethanol to water, and finally placed in distilled water for subsequent staining.
2. Weigert Staining
   1) Preparation of Weigert working solution: Mix Weigert Solution A and Weigert Solution B at a ratio of 1:1 (v/v) to prepare the Weigert working staining solution.
      Note: The Weigert working solution should be prepared freshly before use and used on the same day. The staining performance decreases significantly after 24 h, and further use is not recommended.
   2) Weigert staining: Immerse sections in the freshly prepared Weigert working solution for 3-5 min, then rinse thoroughly with running tap water.
3. Differentiation
   Place the sections in Acidic Ethanol Differentiation Solution for approximately 15 s, then rinse with distilled water for 5–10 min.
4. Fast Green Staining
   Apply Fast Green staining solution to the sections and stain for 1–5 min.
5. Washing and Differentiation
   Quickly rinse the sections with acetic acid solution for 10–15 s to remove excess Fast Green dye, then allow the sections to air dry naturally.
   Note: Alternatively, Acidic Ethanol Differentiation Solution may be used for rapid differentiation for 10–15 s, followed by a brief rinse with tap water.
6. Safranin O Staining
   Apply Safranin O staining solution and stain for 2–5 min.
7. Dehydration
   Dehydrate the sections rapidly with absolute ethanol four times, 3–5 s each time. After the fourth dehydration step, the sections may be examined microscopically. Optimal staining is achieved when cartilage tissue appears red while the background is essentially colorless.
8. Clearing and Mounting
   Clear the sections using xylene, then mount with neutral mounting medium or mounting reagent. Observe under a microscope, and images may be captured for subsequent analysis.

Interpretation of Staining Results

1. Cartilage matrix: red, orange-red, or dark red.
2. Chondrocyte nuclei: blue.
3. Chondrocyte cytoplasm: red.
4. Cytoplasm, muscle, collagen fibers, and osteogenic tissue: gray-green.
5. Other nuclear structures: blue-purple to dark blue.

5 Storage Condition

RT, 1 year.
Protected from light.

5 Precautions

1. For clear visualization of nuclear structures, staining with iron hematoxylin (Weigert's iron hematoxylin) is recommended. This dye provides strong staining intensity, stable coloration, and high contrast, whereas ordinary hematoxylin generally produces weaker staining, which may affect the clear visualization of cell nuclei.
2. Weigert staining solution should not be prepared in advance for long-term storage. It should be freshly prepared according to the specified ratio before use. The prepared working solution generally loses its staining capability significantly after 24 h, and continued use is not recommended.
3. The staining time in Safranin O solution should not be excessively prolonged. Otherwise, a mixed color reaction may occur with the Fast Green stain, causing the tissue to appear bluish-purple or with mixed coloration, which may interfere with the interpretation of staining results.
4. After Safranin O staining, the staining time should not be arbitrarily extended, as this may lead to overstaining, thereby reducing the contrast between cartilage tissue and surrounding background tissue.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.