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Gelatin coating of microscope slides for zebrafish brain tissue sections

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LAPCOM

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Abstract

This protocol describes the preparation of gelatinised glass slides to improve the adhesion of histological sections during routine staining and immunohistochemical procedures. Slides are coated with a thin gelatin film stabilized with chromium potassium sulfate, providing a positively charged surface that enhances tissue adherence and prevents section detachment during extended incubations or multiple washing steps. The method is simple, economical, and suitable for delicate tissues, including zebrafish brain sections.

Guidelines

This protocol is intended to standardise the preparation of gelatinised glass slides to enhance the adhesion of histological sections. The method is suitable for tissues embedded in paraffin, agarose, or OCT medium, as well as for unembedded fresh or fixed tissue sections. It has demonstrated reliable performance across a wide range of section thicknesses, including fine (3–8 µm), semi-thin (10–30 µm), and thicker sections used in specialised applications. Although optimised here for zebrafish brain tissue, the procedure can be readily adapted for specimens from any vertebrate or invertebrate species.

Materials

- Nitrile or latex laboratory gloves
- Safety glasses
- Glass microscope slides
- Drying oven suitable for glass slides
- 600 mL borosilicate beaker
- Magnetic stir bar
- Analytical balance for weighing reagents
- Aluminum foil sheets for weighing chemicals
- Stainless steel or plastic weighing spatulas
- Graduated cylinder (250 and 500 mL capacity)
- Container or staining dish for holding the coating solution
- Slide rack suitable for submerging microscope slides in solution
- Paper filters
- Funnel suitable for hot aqueous solutions
- Funnel stand or support for secure filtration
- Chromium potassium sulfate (chrome alum), powder **Vetec catalog #728**
- Gelatin powder **Vetec catalog #628**
- Dinamicatec D-27 neutral detergent **Dinâmica catalog #1056-5**
- Extran® MA 02 neutral detergent **Merck catalog #107553**

Troubleshooting

Problem

Tissue sections detach during staining or immunohistochemistry

Solution

a) Incomplete triple gelatinisation: ensure that all three coating-and-drying cycles were performed without shortening drying times. b) Insufficient slide cleaning: repeat the detergent cleaning steps and avoid handling slides with bare hands. c) Old coating solution: prepare a fresh batch; solutions older than one month may lose adhesive strength. d) Inadequate drying temperature: confirm that drying occurred at 37–40 °C; lower temperatures reduce cross-linking efficiency.

Problem

Uneven or streaked coating on slides

Solution

a) Solution temperature too low: maintain the gelatin–chrome alum solution at ~40 °C to prevent partial solidification. b) Rapid removal from the solution: withdraw slides slowly to allow uniform drainage. c) Residual moisture on slides before coating: ensure slides are completely dried in the oven before the first gelatinisation cycle. d) Contaminated or poorly filtered solution: filter the solution three times and ensure all equipment is clean.

Problem

Presence of bubbles or uncoated spots

Solution

a) Air bubbles trapped on the glass surface: immerse slides at a slight angle to minimise bubble formation. b) Dust contamination: work in a clean area, keep slides inside the oven or covered when not handling, and avoid leaving coated slides exposed. c) Old or degraded gelatin: use fresh gelatin powder and check for signs of moisture or clumping.

Problem

Slides stick together after coating

Solution

a)Incomplete drying between cycles: ensure each drying phase reaches complete dehydration before the next immersion. b) Insufficient spacing on the rack: space slides adequately to prevent contact during drying.

Problem

Cloudy or precipitated gelatin–chrome alum solution

Solution

a) Overheating: keep temperature at ~40 °C; higher temperatures may denature gelatin. b) Poor filtration: repeat filtration until the solution is visibly clear. c) Old chrome alum stock: replace with fresh reagent.

Safety warnings

Wear appropriate personal protective equipment, including a lab coat, gloves, and safety glasses. Consult the Safety Data Sheets (SDS) for all reagents, particularly for chromium potassium sulfate. Handle hot solutions with care. Collect and dispose of chromium-containing waste according to institutional and environmental regulations.

Before start

Ensure that all glass slides are thoroughly cleaned, grease-free, and completely dry before coating. Prepare a dust-free area for drying the slides and preheat the water bath for dissolving the gelatin–chromium solution. Confirm that the coating solution is homogeneous and free of precipitates immediately before use. This protocol was standardised at LAPCOM (Psychopharmacology and Behaviour Laboratory, UFRGS) for zebrafish brain tissue and is suitable for both routine histological staining and immunohistochemical applications. Although optimised for these tissues, the preparation steps can be applied to a wide range of specimens.

PREPARING THE REAGENTS:

1 The first step is to prepare the solutions required for cleaning the glass microscope slides, followed by the preparation of the gelatin–chromium potassium sulfate (chrome alum) coating solution. All solutions should be prepared using distilled water and handled with appropriate laboratory safety precautions.

1.1 **Dinamicatec D-27 neutral detergent 30%:** to prepare 250 mL of a 30% neutral detergent solution (Dinamicatec D-27):

1.1.1 Using a 250 mL graduated cylinder, measure 75 mL of Dinamicatec D-27 neutral detergent;

1.1.2 Add distilled water to the cylinder until the total volume reaches 250 mL;

1.1.3 Mix gently to ensure homogeneity;

1.1.4 Transfer the solution to a wash bottle or another appropriate container for routine use. This solution may be stored at room temperature.

1.2 **Extran® MA 02 neutral detergent solution (1%):** to prepare 250 mL of a 1% Extran® neutral detergent solution:

1.2.1 Using a 250 mL graduated cylinder, measure 2.5 mL of Extran neutral detergent;

1.2.2 Add distilled water to the cylinder until the total volume reaches 250 mL;

1.2.3 Mix gently to ensure homogeneity;

1.2.4 Transfer the solution to a wash bottle or another appropriate container for routine use. This solution may be stored at room temperature.

1.3 **Gelatin–Chromium Potassium Sulfate Coating Solution (0.06 % chrome alum; 0.6% gelatin in 500 mL):** to prepare 500 mL of the gelatin–chrome alum coating solution:

1.3.1 Weigh 3 g of gelatin powder and transfer it to a 600 mL borosilicate beaker;

1.3.2 Weigh 0.3 g of chromium potassium sulfate (chrome alum) using aluminium foil as a weighing surface, and transfer to the same beaker;

1.3.3 Add 500 mL of distilled water to the beaker;

1.3.4 Place the beaker on a magnetic stirrer and heat the solution to 40 °C ;

1.3.5 Stir the mixture gently until all solids are fully dissolved and the solution becomes clear;

1.3.6 Filter the solution three times through paper filters using an appropriate funnel and funnel support;

1.3.7 Transfer the filtered solution to a clean, appropriately labelled container.

Storage: Store the solution at 4 °C . Reheat to 40 °C before use to ensure complete liquefaction.

STEP-BY-STEP PROCEDURE 8h 47m

2 STEP-BY-STEP PROCEDURE

2.1 Cleaning of Glass Slides 45m

2.1.1 Place the glass microscope slides in a suitable container and cover them completely with the 30% Dinamicatec D-27 neutral detergent solution;

2.1.2 Allow the slides to soak for at least 00:30:00 ;

2.1.3 Rinse thoroughly with running tap water to remove all detergent residues;

2.1.4 Submerge the slides in the 1% Extran solution for 00:15:00 ;

2.1.5 Rinse again under running tap water, followed by a final rinse with distilled water;

2.1.6 Place the slides vertically on a rack and dry them in a laboratory oven until all moisture is fully removed. Avoid exposing the slides to dust during handling.

2.2 Coating of Slides with Gelatin–Chrome Alum Solution 8h 2m

2.2.1 First Gelatinisation

2.2.1.1 Warm the gelatin–chrome alum solution to 40 °C to ensure complete liquefaction;

2.2.1.2 Pour an appropriate volume into a clean container or staining dish;

2.2.1.3 Immerse the slides using a slide rack, ensuring each slide is fully submerged and evenly coated;

2.2.1.4 Maintain immersion for 00:02:00 ;

2.2.1.5 Remove the slides slowly to allow excess solution to drain uniformly;

2.2.1.6 Place the coated slides vertically on a clean drying rack;

2.2.1.7 Air-dry in a dust-free environment for at least 02:00:00 ;

2.2.1.8 Transfer to a drying oven at 40 °C for 06:00:00 or overnight.

2.2.2 Second Gelatinisation Cycle

Repeat steps 2.2.1.1 to 2.2.1.8, to produce a second coating layer.

2.2.3 Third Gelatinisation Cycle

Repeat the same coating and drying sequence (2.2.1.1 – 2.2.1.8) a third time. This completes the triple gelatinisation, providing robust slide adhesion for demanding staining protocols.

Note

Once fully dried after the third cycle, store the slides in a clean, dry slide box or sealed container protected from dust.

CRITICAL NOTES:

3 ▪ Any residual grease, dust, or detergent will interfere with coating uniformity and reduce tissue adhesion. Handle slides only by the edges.

- Lower temperatures may cause partial solidification, leading to uneven coating; higher temperatures may degrade the gelatin.
- Skipping or shortening cycles will compromise adhesion, particularly for thick sections or protocols involving multiple washing steps.
- Air bubbles on the glass surface will create uncoated areas that result in tissue detachment during staining or immunohistochemistry.
- Dust contamination at any stage will permanently embed particles into the coating film.
- Use clean containers and fresh filtering materials for each preparation to maintain consistency across batches.
- Exposure to humidity or dust during storage will weaken the adhesive properties of the triple-gelatinised surface.