Immunohistochemical detection of IGF-II in paraffin embedded sections of fetal rat tissues

Introduction

Insulin-like Growth Factor-II (IGF-II) is produced by many tissues in the body, especially during fetal development. Immunohistochemical detection of IGF-II is one of the important tools for studying the local expression of IGF-II peptide in tissues. This document details the ExtrAvidin-Cy3 staining protocol for IGF-II detection in paraffin sections of rat embryonic tissues using GroPep Bioreagents' IGF-II antibody for immunohistochemistry. In this protocol, the paraffin-embedded fixed tissue section is de-paraffinized, re-hydrated, treated for antigen retrieval, trypsinized, incubated with the primary antibody, followed by a biotinylated anti-rabbit immunoglobulin (secondary antibody). The specifically bound secondary antibody is then visualized by ExtrAvidin conjugated Cy3.

GroPep Bioreagents IGF-II antibody for immunohistochemistry, affinity purified (Catalogue # PAAL1) has been tested in embryonic rat tissues. The antibody does not recognize rat IGF-I in enzyme immunoassays or in Western blotting. Pre-absorption of the antibody (diluted 1:100) for 1 hour at room temperature with 1-2 mg human or rat IGF-II abolishes the immunohistochemical staining in fetal rat sections.

Equipment and Reagents Required

Equipment

1. Solvent tanks

2. Slide racks to fit into the solvent tanks

3. Humidified chamber or airtight plastic container

4. Snowcoat X-TRA slides (Surgipath SP-00210) or similar.

5. Coverslips

6. Pipettes and other general laboratory equipment

7. Horizontal shaker

8. UV microscope

Reagents

1. GroPep Bioreagents IGF-II antibody for immunohistochemistry (Catalog # PAAL1)

2. GroPep Bioreagents human IGF-II (Catalogue # FU020) or rat IGF-II (Catalog # AU020) for 20 µg vial sizes.

3. Dissolve GroPep Bioreagents IGF-II antibody for immunohistochemistry in 500 µl distilled or Milli-Q water (a 1:10 dilution). Make up dilutions of the antibody (suggested final dilution of 1:100) in 1% BSA/PBS as required, and mix thoroughly. Dispense the remaining antibody into convenient aliquots and store at - 20°C.

Avoid repeated freeze-thawing of the diluted antibody.

3. Biotinylated goat anti-rabbit IgG (Sigma B8895). Dilute 1:1000 in 1% BSA/PBS.

4. ExtrAvidin conjugated Cy3 (Sigma E4142). Dilute 1:200 in 1% BSA/PBS.

5. Target Retrieval Solution (Dako S1700).

6. Trypsin (Sigma T7409).

7. Fluorescent Mounting Media (Dako S3023).

8. Phosphate-buffered saline (PBS): 10 mM, pH 7.4, 150 mM NaCl.

9. Blocking Solution (10 % BSA/PBS). Dissolve 1 g bovine serum albumin (BSA) (Sigma A7888) in 10 ml PBS with gentle stirring.

10. 1 % BSA/PBS: Dissolve 1 g bovine serum albumin (BSA) (Sigma A7888) in 100 ml PBS with gentle stirring. Use this solution to dilute antibodies.

Protocol

Unless otherwise specified carry out all procedures at room temperature. A humidified chamber is required for some of the incubation steps. Wipe slides around sections and remove most of the liquid from tissues before next incubation. Avoid drying of specimens between steps. Use 50 - 100 µl or sufficient reagent to cover the sections for blocking or antibody incubations. Use 3-5 ml PBS for each slide washing.

1. Select the tissue sections desired for immunohistochemistry. Fetal rat sections can be used as a positive control. We routinely use 6 - 7 µm paraffin sections mounted on Snowcoat X-TRA slides.

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2. De-paraffinize sections twice, 10 minutes each in 100 % xylene.

3. Hydrate sections with 100 % ethanol for 2 minutes, and then with 75 %, 50 %, 25 % (all v/v) ethanol for 2 minutes each.

4. Soak sections in distilled or Milli-Q water for 2 minutes.

5. Antigen Retrieval: Put slides into a Microwave proof ladle. Place ladle into a Microwave proof container and make sure all slides are covered with Target Retrieval Solution. Microwave slides as follows:

i) Microwave 2 min on high (650W); top-up level of Target Retrieval Solution in slide container between each microwave step if necessary. Swirl solution in container to re-mix.

ii) Microwave 5 min on medium-high, re-mixing and topping up with Target Retrieval Solution if necessary.

iii) Microwave 5 min on medium.

6. **IMPORTANT**: Place microwave container in ice to allow tissue sections and Target Retrieval Solution to cool to at least 40°C before proceeding with Step 7.

7. Place slides into Trypsin solution at 37°C for 3 min (0.625 g trypsin in 250 ml PBS).

8. Wash sections with PBS five times, shaking off excess PBS between each wash.

9. Carefully wipe around the sections to absorb excess moisture.

10. Incubate sections with blocking solution (10% BSA/PBS; 100 µl per section) for 60 minutes at room temperature in a sealed humid chamber.

11. Dilute GroPep Bioreagents IGF-II antibody for immunohistochemistry 1:10 in 1% BSA/PBS (giving a final antibody dilution of 1:100). Add 100 µl to each slide and incubate overnight at 4°C in a sealed humid chamber.

12. Wash sections with PBS five times, shaking off excess PBS between each wash.

13. Add 100 µl biotinylated goat anti-rabbit IgG (diluted (1:1000 in 1% BSA/PBS) to each slide. Incubate 1 hour at room temperature.

14. Wash sections with PBS five times, shaking off excess PBS between each wash.

15. Add 100 µl ExtrAvidin conjugated Cy3 solution to each slide. Incubate 40 min at room temperature in a sealed humid chamber. Keep sections out of light during and following this incubation.

16. Wash sections in PBS five times, shaking off excess PBS between each wash.

17. Cover each section with 1 drop of Fluorescent Mounting Solution. Add coverslip. Store slides at 4°C overnight to allow mounting medium to set before viewing sections in a UV microscope, using a green filter.

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