

## Introduction

Insulin is a hormone secreted in the pancreas by the  $\beta$  cells of the Islets of Langerhans. Insufficient levels of insulin in the body can result in diabetes. This protocol enables specific gold-labeling of insulin in insulin containing cells.

### ➤ The reagents required:

- PBS
- 1% BSA in PBS, pH 8.2 (for example Sigma, Cat. No. P3688)
- Double distilled water (ddH<sub>2</sub>O)
- Unmasking reagent (for example Auto/Zyme<sup>®</sup>, Biomedica Corp., Cat. No. M34)
- Blocking agent: Normal Goat Serum (NGS) (for example Jackson ImmunoResearch, Cat. No. 005-000-121)
- Primary antibody: anti-pig insulin (for example Sigma, Cat. No. I 8510)
- Antibody Diluent Solution (for example Zymed, Cat. No. 00-31118)
- Tween<sup>®</sup> 20 (for example Sigma Cat. No. P-7949)
- Secondary antibody: Goat anti guinea pig IgG (H&L)-ultra small (for example Aurion, Cat. No. 800.144 )
- Silver enhancement kit: R-GENT SE-EM ( Aurion, Cat. No. 500.033)
- Uranyl acetate (for example EMS , Cat. No. 22400 ), 5% stock in double distilled water, pH 3.5 with HCl, kept at 4°C in the dark.

### ➤ Procedure:



#### **NOTE**

- a. Pancreas tissue should be fixed with formalin before the immunolabeling procedure.***
- b. Silce the tissue to the smallest possible size (400-800 micron) for easier handling and good penetration.***

1. Wash tissue three times in PBS for 5 minutes to remove excess aldehyde groups.
2. Incubate tissue with unmasking reagent for 5 minutes, follow the manufacturer's recommendations.
3. Wash tissue three times in PBS, 5 minutes each wash.
4. To avoid non-specific background, incubate tissue in 5% NGS/1% BSA in PBS, pH 8.2, for 30 minutes.

Doc. No.: QXP008	V 1.0	<b>Title: Insulin Immuno-gold Labeling</b>
Written by: Anya Vainshtein	Approved by: Barak Lavi	Date of Issue: 04/05/2005

5. Incubate with primary antibody: anti-pig insulin, diluted in antibody diluent solution (1:1000) for 2 hours.
6. Wash tissue three times in 0.05% Tween 20/1% BSA in PBS, pH 8.2, 5 minutes each wash.
7. Incubate with secondary antibody, goat anti guinea pig IgG(H&L)-ultra small, diluted in antibody diluent solution (1:300) for 1 hour.
8. Wash tissue three times in 0.05% Tween<sup>®</sup> 20/1% BSA in PBS, pH 8.2, 5 minutes each wash.
9. Wash tissue three times in PBS, 5 minutes each wash.

#### **Silver enhancement:**

10. Wash tissue five times in double distilled water, 5 minutes each wash.
11. Incubate in fresh silver enhancement solution for 1.5 hours.
12. Wash tissue five times in double distilled water, 5 minutes each wash.

#### **Uranyl acetate staining:**

13. Incubate with 0.05% Uranyl Acetate for 5 minutes.
14. Wash tissue five times in double distilled water, 5 minutes each wash.



#### **NOTE**

*Protocols provided by QuantomiX are based on work conducted at QuantomiX laboratories. They are given as a starting point which will facilitate the user's first steps in acquiring the desired imaging results. It is the user's responsibility to determine the suitability of any protocol published by Quantomix to their applications. Users may find it necessary to modify protocols in order to obtain the information required for their study.*