

Application Note

Leica EM IGL – Rat pancreatic tissue

Courtesy of: George Posthuma
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Utrecht, Netherlands

Living up to Life

Leica
MICROSYSTEMS

Leica EM IGL Application Note

Rat pancreatic tissue

Perfusion fixed with 2% paraformaldehyde, 0.2% glutaraldehyde in phosphate buffer for 2 hours.

After excision the tissue was embedded in 12% gelatine in phosphate buffer.
Appropriate blocks were cut and infiltrated overnight with 2.3 M sucrose.
The blocks were mounted on aluminium specimen holders and frozen in liquid nitrogen.

Ultrathin frozen sections (50 nm) were cut on a Leica UCT with FCS at -120°C .
Transferred to a 100 mesh Formvar-carbon coated nickel grid.

Immuno-incubation was performed with Rabbit ant rat amylase serum diluted 1:50 in PBS/albumin 1%.

The antibodies were visualized with protein A- Gold 10 nm (OD 0.2).

Final contrasting:
1.8% methylcellulose/0,4 % uranyl acetate.

Literature:

Geuze HJ, Slot JW, van der ley PA, Scheffer RC.

Use of colloidal gold particles in double-labelling immunoelectron microscopy of ultrathin frozen tissue sections.

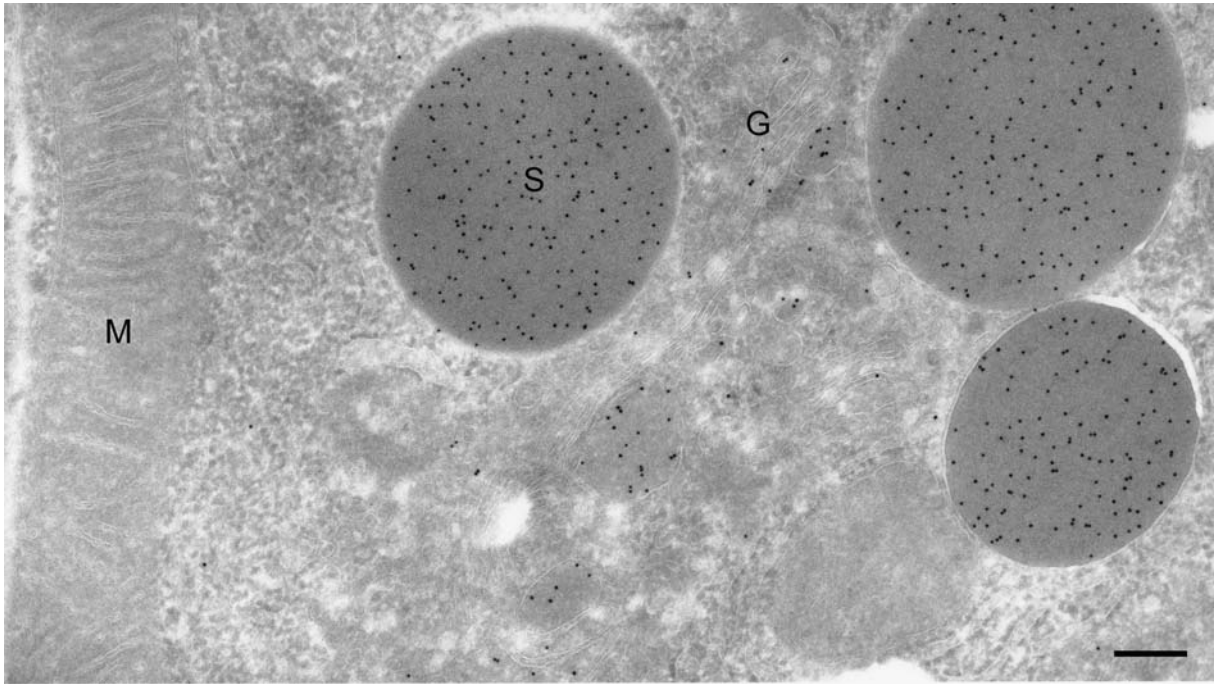
J Cell Biol. 1981 Jun;89(3):653-65

Posthuma G, Slot JW, Geuze HJ.

Immunocytochemical assays of amylase and chymotrypsinogen in rat pancreas secretory granules.

Efficacy of using immunogold labelled ultrathin cryosections to estimate relative protein concentrations

J Histochem Cytochem. 1984 Oct;32(10): 1028-34



Rat pancreas.
Immuno-incubation performed with Rabbit ant rat amylase serum.
The antibodies were visualized with protein A- Gold 10 nm.

S = secretory granule
G = Golgi complex
M = mitochondrion.
Bar = 200 nm

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Leica EM IGL Program Description



Date:	18.07.2003
Operator:	G. Posthuma
Experiment no:	1
Program no:	1
Program name:	Pancreas

Slide no	Reagent	Drop size (µl)	Time (min)
1	PBS/Gly	30	5
2	PBS/Gly	30	5
3	PBS/alb (1%)	30	5
4	Prim. Antibody	5	30
5	PBS	30	5
6	PBS	30	5
7	PBS	30	5
8	PBS/alb (1%)	30	5
9	Protein A-gold	30	30
10	PBS	30	5
11	PBS	30	5
12	PBS/glutar*	5	1
13	PBS	30	5
14	Water	30	5
15	Water	30	5
16	Water	30	5
17	Uranyl oxelate	30	5
18	Water	30	1
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Slide no	Reagent	Drop size (µl)	Time (min)
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Remarks:

Outside the IGL:

MC/uranyl acetate 50µl / 1min.

MC/uranyl acetate 50µl / 5min.

*Wet the filter paper in the side container with PBS/glycine or Tris to prevent fixative vapors entering other containers (in particular those with immunoreagents, they will be fixed!)