

Application Note

Leica EM IGL –
Rat liver embedded in LR white resin
Immuno labelling of Catalase
Detected with Goat anti-rabbit

Courtesy of: Bruno M. Humbel
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Living up to Life

Leica
MICROSYSTEMS

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IGL Test Run

Sample

Rat liver fixed with 2% formaldehyde and 0.02% glutaraldehyde in PBS. After PBS wash small liver pieces of about 1 mm³ were cut and dehydrated in a graded series of ethanol. Thereafter, they were cut embedded into LR white resin. Sections of about 80nm were cut and collected on Pioloform/carbon coated nickel grids (H100).

Primary Antibodies

Rabbit anti-catalase from Rockland (10 mg/ml, IgG fraction, bovine liver; 200-4151, lot# 7234)
<http://www.rockland-inc.com/cgi-bin/gen.cgi?page=/spec/200-4151.html>

The primary antibody is used at a concentration of 40µg/ml

Secondary Antibodies

Goat anti-rabbit	IgG ultra-small	GAR GP-US	GAR-90512/1	11/2000
	IgG 15nm	GAR 15nm	GAR-30113/2	07/2004
	IgG 10nm	GAR 10nm	GAR-10710/3	01/2003

The gold antibodies are from Aurion <http://www.aurion.nl/>. The ultra-small gold particles (US) are used at a dilution of 1/100 and the larger gold particles at a dilution of 1/10.

Chemicals and solutions

PBS pH 7.4

137.	mM NaCl	8.	g/l
2.7	mM KCl	0.2013	g/l
8.1	mM Na ₂ HPO ₄	1.1499	g/l
1.5	mM KH ₂ PO ₄	0.2041	g/l

TBS pH 7.6

137.	mM NaCl	8.	g/l
2.7	mM KCl	0.2013	g/l
50	mM TRIS/HCl	1.1499	g/l

Labelbuffer (TBG)

TBS pH 7.6

0.5% (w/v) BSA (fraction V)

0.045% (w/v) Teleostean gelatin

Sigma A-4503

Sigma G-7765

Fixative

1% (v/v) glutaraldehyde in PBS pH 7.2

Staining

2% (w/v) uranyl acetate in H₂O

Merck 8473

0.4% lead citrate according to Venable & Coggeshall (J Cell Biol, 25, 407, 1965)

Silver enhancement

HQ Silver Nanoprobes 2012 <http://www.nanoprobes.com/>

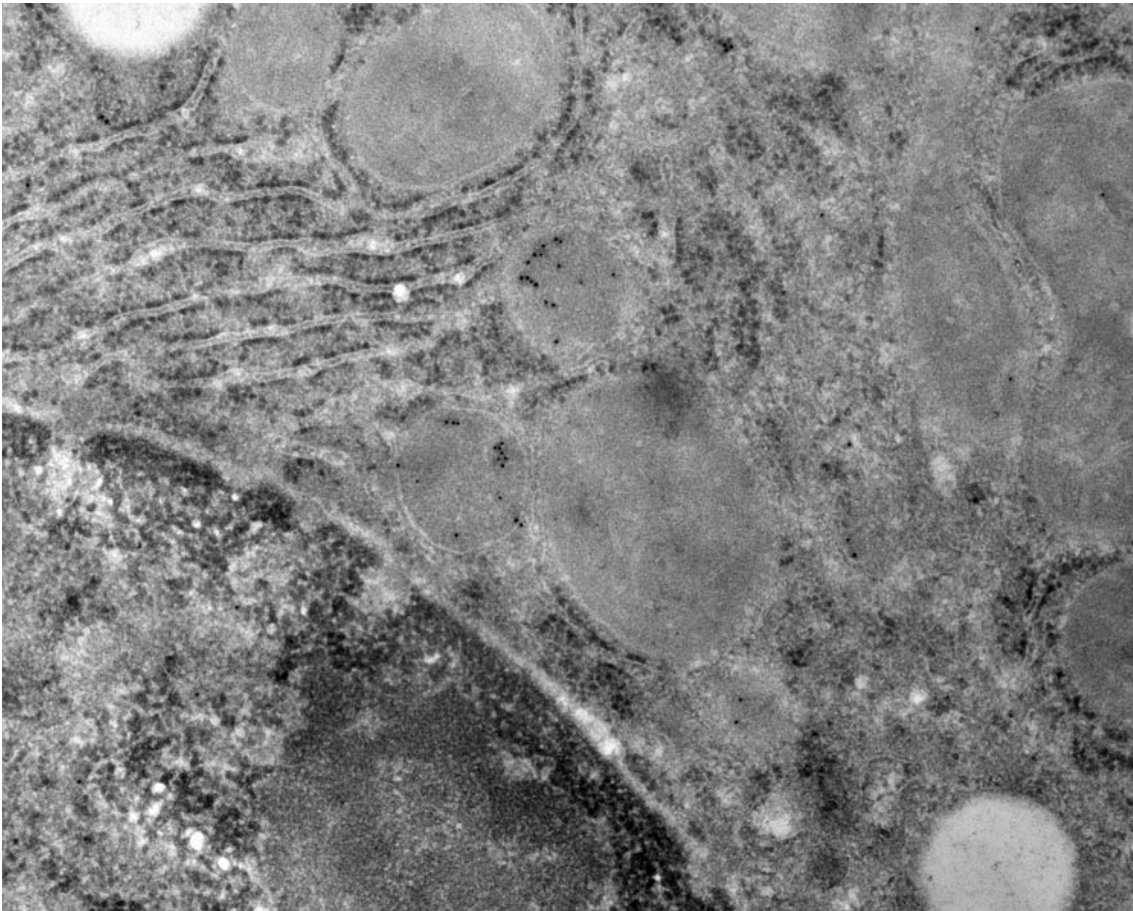
Note: (Silver-enhancement, 5 min, and lead citrate staining, 1 min, was done on parafilm, not in the IGL)

Leica EM IGL Program Description

Date	27.Oct.03
Operator	Bruno Humbel
Experiment no	U266_IGL
Program no	98
Program name	Bruno's standard

Slide no	Time (min.)	Reagent	Drop size (µl)
1	5	TBS	30
2	5	TBG	30
3	5	TBG	30
4	60	primary antibody	5
5	5	TBG	30
6	5	TBG	30
7	5	TBG	30
8	5	TBG	30
9	5	TBG	30
10	5	TBG	30
11	60	secondary antibody	5
12	5	TBG	30
13	5	TBS	30
14	5	PBS	30
15	5	PBS	30
16	5	PBS	30
17	5	PBS	30
18	10	glutaraldehyde	30
19	5	bidest	30
20	5	bidest	30
21	5	bidest	30
22	5	bidest	30
23	5	uranyl acetate	5
24	2	bidest	30
25			
26			
27			
28			

Slide no	Time (min)	Reagent	Drop size (µl)
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