

Application Note

Leica EM IGL – Immuno Labelling of Catalase on Liver Sections

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Immuno Labelling of Catalase on Liver Sections

IGL Run

Sample

Rat liver fixed with 2% formaldehyde and 0.02% glutaraldehyde in PBS. After PBS wash small liver pieces of about 1mm³ were infiltrated with 12% gelatine/PBS at 37°C. After incubation over night in 2.3 M sucrose the blocks were mounted on the stubs for the FCS. Sections of about 90 nm were cut at -120°C and collected on Pioloform/carbon coated nickel grids (H75.) The sections were stored over night with the picking- up sucrose drop on the grids at 4°C.

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 ultra-small	GAR GP-US	GAR-80225/1	08/1999
	IgG 15nm	GAR 15nm	GAR-30113/2	07/2004
	IgG 15nm	GAR 15nm	GAR-00605/2	12/2001
	IgG 10nm	GAR 10nm	GAR-10710/3	01/2003
	IgG 6nm	GAR 6nm	GAR-21206/1	06/2004
	IgG 15nm	GAR 15nm	GAR-90722/1	04/2001

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)

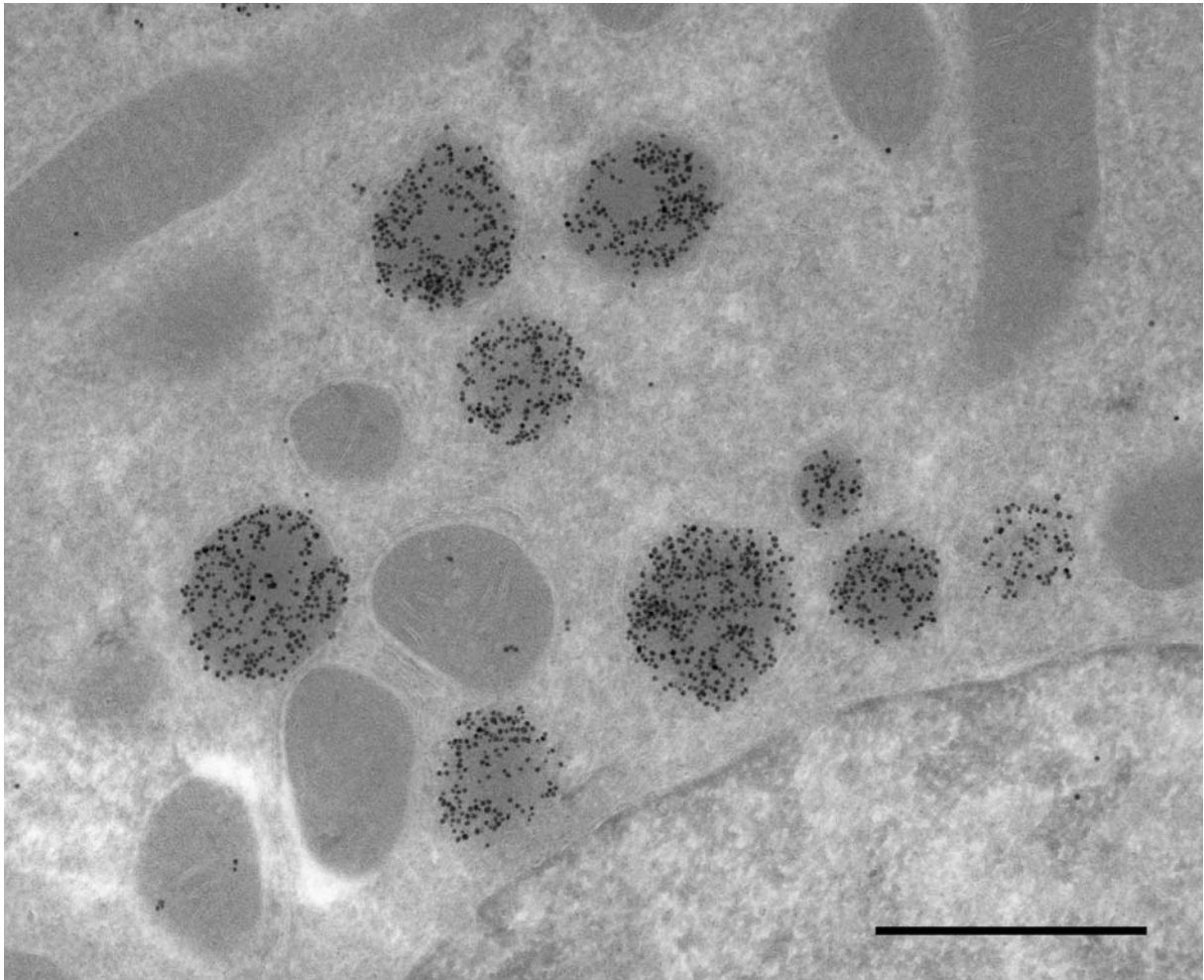
TBS pH 7.6

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

0.045% (w/v) Teleostean gelatin

Sigma A-4503

Sigma G-7765



Rat liver,
peroxisomes labelled with rabbit anti-catalase antibodies.
Detected with goat anti-rabbit ultra small gold particles.
Silver enhanced with R-Gent SE-EM.
Bar = 1µm

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