

Application Note

Leica EM IGL – HepG2 – human hepatoma cell line

Courtesy of: Prof. Torrisi,
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Living up to Life

Leica EM IGL Application Note

Human hepatoma cell line

Tissue type:

HepG2 – human hepatoma cell line

Pre preparation protocol:

Cells were fixed in 2% paraformaldehyde, 0.2% glutaraldehyde in phosphate buffer for 2 h and embedded in 12% gelatin.

Blocks were cut and infiltrated overnight with 2.3 M sucrose before freezing in liquid nitrogen.

Ultrathin cryosections were obtained using a Leica UCT with FCS.

Labelling:

Monoclonal mouse anti-human

Diluted in PBS/BSA 1%

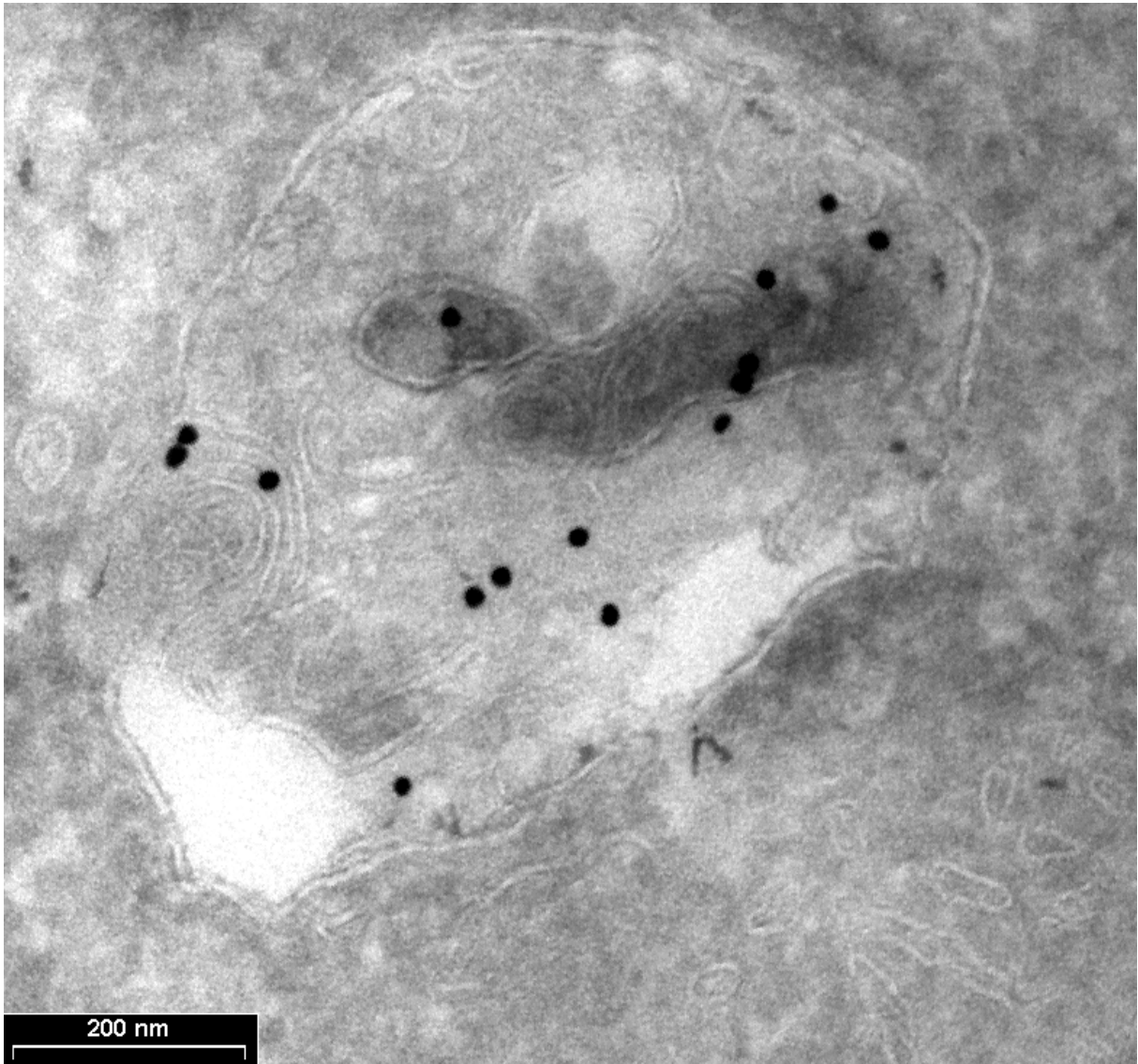
Visualised with PAG 18nm

Bar = 200 nm

Labelled for Cathepsin D

(Cathepsin D is a normal lysosomal proteinase with is expressed in a cell.

Cathepsin D plays an important role in malignant transformation an may be a useful prognostic indicator for breast cancer).



Cryo sectioning of HepG2 – hepatome.
Labelled for Cathepsin D.
Monoclonal mouse anti-human diluted in PBS/BSA 1%.
Visualised with PAG 18nm.

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Immunoreagent1:

Anti-cathepsin D monoclonal antibody diluted in PBS1%BSA.

Immunoreagent2:

Secondary antibodies in 1%BSA with protein A/colloidal gold coniugate in PBS1%BSA.

Outside the IGL:

Staining Meth. Cellulose/Uranyl ac. 4% for 10-15 min.

HepG2

Program 03 (Slides 1-22)



Duration: 2 hours, 56 minutes.

Slides	Drop size	Duration	Pause/Cont.
Slide 01: PBS-gly	30 ul	00:02	Cont.
Slide 02: PBS-gly	30 ul	00:02	Cont.
Slide 03: PBS-gly	30 ul	00:02	Cont.
Slide 04: PBS-gly	30 ul	00:02	Cont.
Slide 05: Immunoreag.1	5 ul	01:00	Cont.
Slide 06: PBS/BSA	30 ul	00:02	Cont.
Slide 07: PBS/BSA	30 ul	00:02	Cont.
Slide 08: PBS/BSA	30 ul	00:02	Cont.
Slide 09: PBS/BSA	30 ul	00:02	Cont.
Slide 10: Immunoreag.2	5 ul	01:00	Cont.
Slide 11: PBS/BSA	30 ul	00:02	Cont.
Slide 12: PBS	30 ul	00:02	Cont.
Slide 13: PBS	30 ul	00:02	Cont.
Slide 14: PBS	30 ul	00:02	Cont.
Slide 15: PBS	30 ul	00:02	Cont.
Slide 16: Glutar	5 ul	00:05	Cont.
Slide 17: PBS	30 ul	00:02	Cont.
Slide 18: Water	30 ul	00:02	Cont.
Slide 19: Water	30 ul	00:02	Cont.
Slide 20: Water	30 ul	00:02	Cont.
Slide 21: Water	30 ul	00:02	Cont.
Slide 22: Water	30 ul	00:02	Cont.