Leica EM IGL

Automated Immunolabelling System
according to Posthuma

Leica
MICROSYSTEMS

Immunolabelling, tedious in the past …

With the introduction of particulate immuno-markers in the 1970's and its revolution in 1978 when Roth used colloidal gold particles as a marker, a new era began in the localization of the constituents of the cell. Immunogold labelling became the method of choice for detection of antigens in biological samples.

Until today, immunogold labelling of ultrathin sections on grids has been carried out completely by hand.

- Drops of different reagents are arranged in a highly specific order on a very clean surface.
- Each grid is carefully transferred from one drop to the other – by hand.
- The sequence of grid transfer must be controlled very carefully to avoid mistakes.
- The duration of each immunoreagent and wash step must be carefully noted to achieve reproducible results.

But why not reduce your effort?

- Why spend many hours in front of a Parafilm sheet?
- Why risk losing grids/sections while transferring them from one drop to the other?
- Why risk ruining a complete run by transferring a grid to the wrong droplet?
- Why risk cross contamination by contaminated forceps or loop?
- Why have results with low reproducibility?
- Why not turn this into an automatic process?

… routine today!

LEICA EM IGL Designed by Werner Hölbl

The LEICA EM IGL meets the demands of today's immunolabelling laboratory.
By preparing all your reagents together, you can set up a run within a few minutes and free your time for more productive activities. Reagent droplets are placed in humidified carries and moved automatically to grids according to the programme in use.

The Leica EM IGL gives you

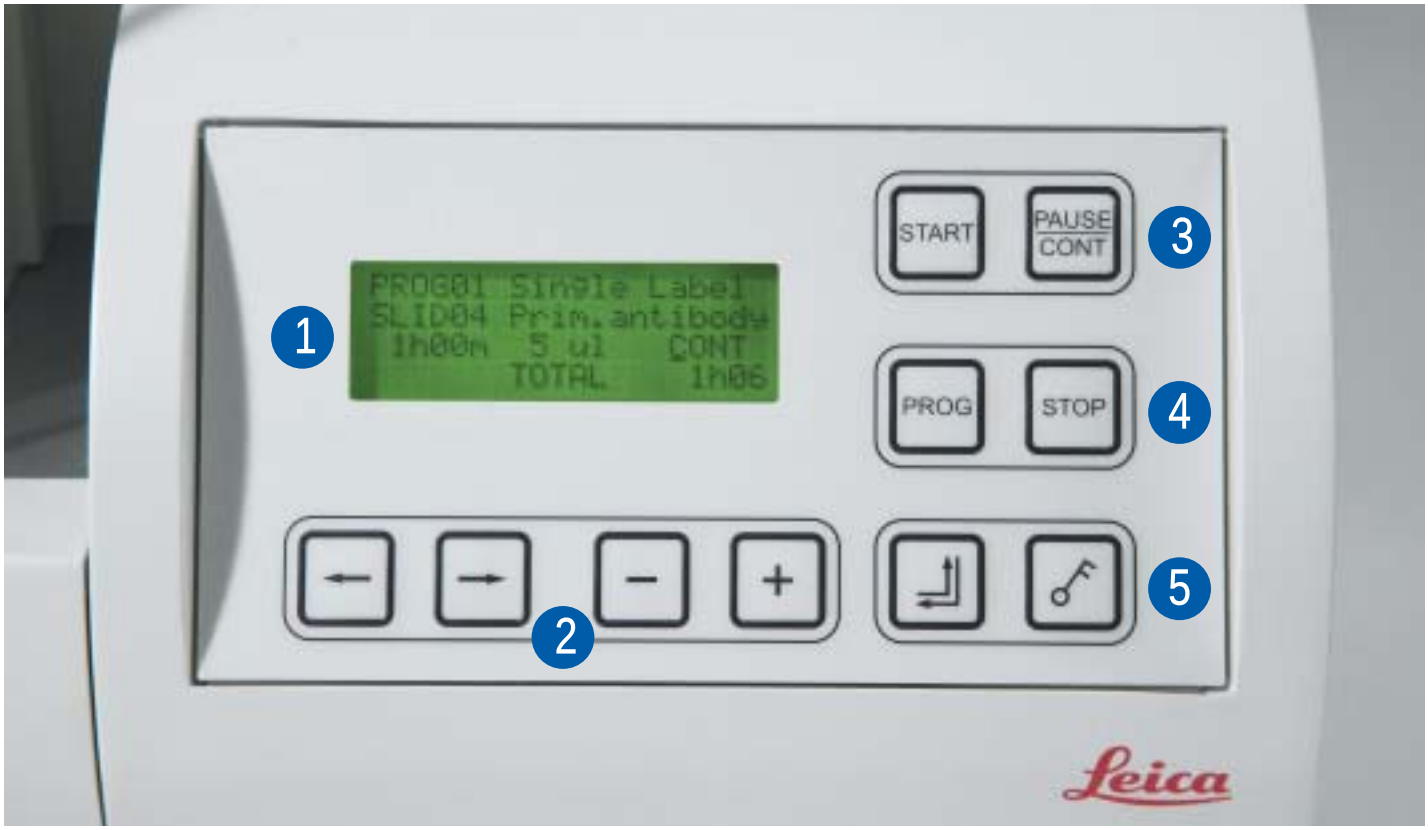
- Labelling of 24 grids simultaneously.
- The ability to use up to 24 different primary antibodies in one run.
- 80% time saving compared to the manual method.
- Cost saving - you are free for more productive activities.
- Specimen safety – the correct sequence of reagents will always be applied.
- Minimising cross contamination from forceps and loops during labelling.
- Reproducible results from each immunolabelling run by following the exact incubation times according to your protocol.
- The possibility of standard contrasting with lead citrate and uranyl acetate.

From easy programming …

Latest technology and PC programming software is used to interface user and instrument.
A simple to use control panel allows data storage of

- Up to 99 programmes.
- All of which can be named.
- Reagents can be selected from a reagent list which can be customised by the user.

Programming is carried out either via the membrane-covered keyboard or PC.
Audible warnings and clear hints are given to allow simple programming.
Intelligent software allows labelling to continue after a power failure.



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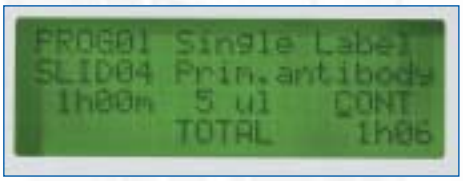
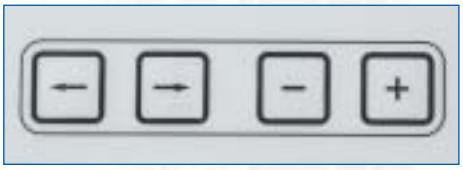


Display
(The example shows the screen during programming).

- Programme number and name
- Slide number and name
- Duration time, droplet size and continue (CONT) or PAUSE before the next slide
- Total time of programme

2



Selection and adjusting of programmes

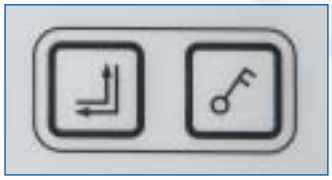
3

Keys to start and pause a programme

4

Selecting a programme
Interrupting a programme

5



Manual movement of a slide
Keyboard lock-function

… via easy set up …

Load up to 24 grids onto the magnetic holder
Load droplets onto slides
Load into EM IGL
Press START

Logical droplet management pads provide clear control over the droplet and grid arrangement, thus preventing any user error. A special Teflon-coated grid holder accepts up to 24 nickel grids and ensures the back of the grid remains absolutely dry.

Date	02.09.2003
Operator	
Experiment no	
Program no	
Program name	

Gridholder	Pos.	Specimen
	1 a	MDCK Cells
	1 b	MDCK Cells
	1 c	MDCK Cells
	2 a	MDCK Cells
	2 b	MDCK Cells
	2 c	MDCK Cells
	3 a	MDCK Cells
	3 b	MDCK Cells
	3 c	MDCK Cells
	4 a	Rat pancreas
	4 b	Rat pancreas
	4 c	Rat pancreas
	5 a	Rat pancreas
	5 b	Rat pancreas
	5 c	Rat pancreas
	6 a	Rat pancreas
	6 b	Rat pancreas
	6 c	Rat pancreas
	7 a	Rat liver
	7 b	Rat liver
	7 c	Rat liver
	8 a	Rat liver
	8 b	Rat liver
	8 c	Rat liver

Loading the grids onto the holder in the correct position according to the droplet management pad.

Special ‘24 well’ slides hold the reagent drops in exactly the correct position in relation to the grids. Immunoreagents are 5 µl in volume and rinse steps are 30 µl. For quick addition of wash and buffer droplets to the slides, a multi 8-channel pipette is provided. A single channel pipette is used for adding the valuable antibody drops.

Using a multi 8-channel pipette for fast and accurate addition of wash and buffer droplets.

Leica EM IGL

Droplet Management Pad

Slide	Pos.		Immuno Reagent 1	Immuno Reagent 2
	1	a	MAB CD 63	Rabbit anti mouse
		b	MAB CD 63	Rabbit anti mouse
		c	MAB CD 63	Rabbit anti mouse
	2	a	Rx M MHC II	
		b	Rx M MHC II	
		c	Rx M MHC II	
	3	a	RxM MHC 1	
		b	RxM MHC 1	
		c	RxM MHC1	
	4	a	RxRat Amylase	
		b	RxRat Amylase	
		c	----------	
	5	a	Rab 17	
		b	Rab 18	Rabbit anti mouse
		c	Rab 19	Rabbit anti mouse
	6	a	Rab 20	Rabbit anti mouse
		b	Rab 21	Rabbit anti mouse
		c	Rab 22	Rabbit anti mouse
	7	a	MAB ASGP 1/60	Rabbit anti mouse
		b	MAB ASGP 1/60	Rabbit anti mouse
		c	----------	Rabbit anti mouse
	8	a	MFR 46 1/50000	
		b	MFR 46 1/50000	
		c	MFR 46 1/50000	

Using a single channel pipette to add the antibody drops.

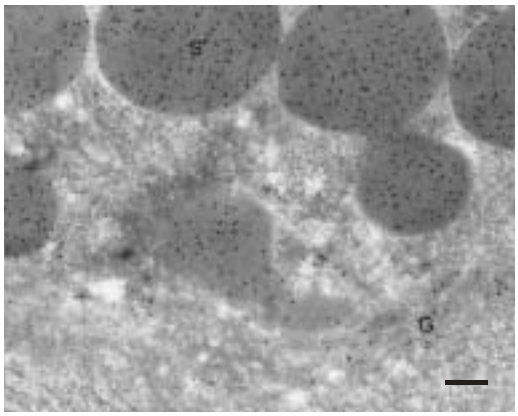
… and easy loading …

Loaded grid holder with grids facing down is placed into the grid station of the LEICA EM IGL.

Beginning with the first reagent according to your programme the stack of the LEICA EM IGL is loaded with the slide carriers.

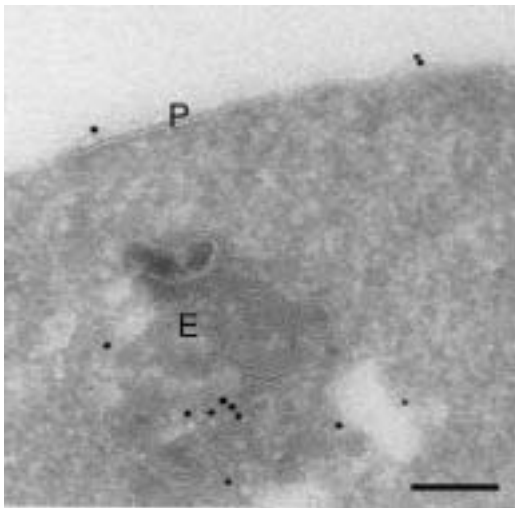
Once the slides and grids are loaded to the LEICA EM IGL and START button is pressed **YOU can carry out more important things than moving grids from one drop to the next.**

… to perfect results on cryo sections …



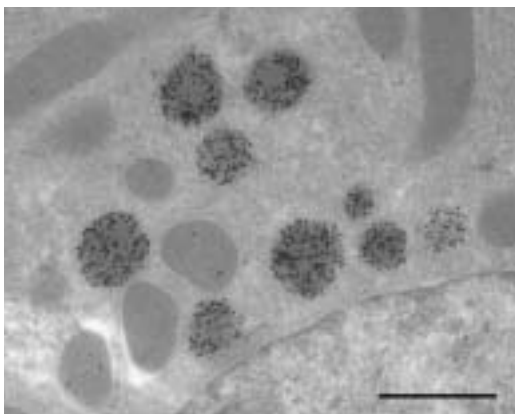
Rat pancreas.
Bar = 200 nm
S = Secretory granule
G = Golgi complex
R = Rough endoplasmatic reticulum

Courtesy of: George Posthuma
University Medical Centre Utrecht, Netherlands



HELA cell.
Incubated with cholesterol
marker coupled to biotin.
Bar = 200 nm
P = Plasma membrane
E = Endosomes/lysosome

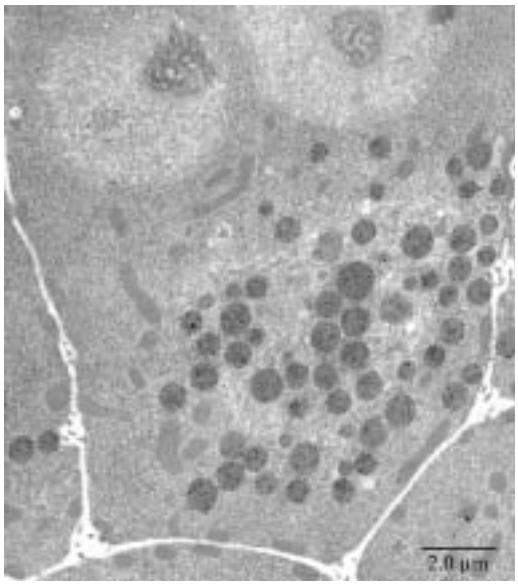
Courtesy of: George Posthuma
University Medical Centre Utrecht, Netherlands



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 = 1 µm

Courtesy of: Bruno M. Humbel
Molecular Cell Biology
Faculty of Biology,
Utrecht University,
Netherlands

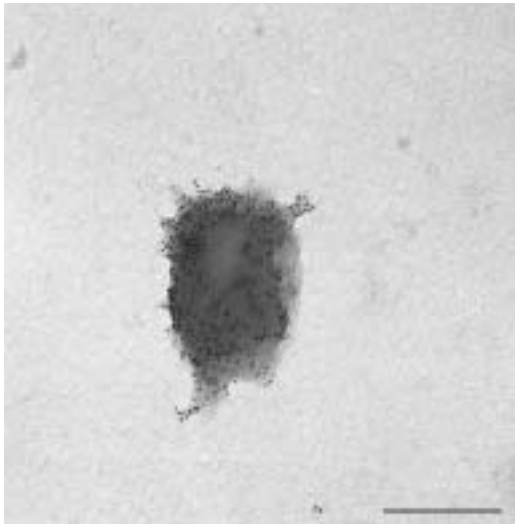
… and perfect results on resin sections …



Mouse pancreas
Cryo fixed followed by freeze substitution and low temperature embedded in HM20. Immunogold/silver labelling of alpha-amylase using Aurion Ultrasmall gold conjugate and R-gent SE-EM silver enhancement reagent.
Bar = 2 µm

Courtesy of: Hong Yi, Emory School of Medicine
Microscopy Core, Emory Univ., Atlanta, USA.

… to viruses on coated grids.



Sucrose gradient purified Modified Virus Ankara (MVA).
The B5R envelope protein is labelled with a rabbit anti-B5R antibody and detected with a goat anti-rabbit antibody coupled to 6 nm gold particles.
Bar = 200 nm

Courtesy of: Daniele Spehner
INSERM, Strasbourg, France.

Technical Specifications:

Dimensions:

Width: 50 cm
Depth: 26 cm
Height: 54 cm
Weight: 16 kg

Electrical power consumption: 50 W
Wide range power supply for 100 – 240 VAC, 50 – 60 Hz.
Power is via standard socket. No special room requirements are needed.

Set up:

With the order number 70 89 01 a complete working outfit with all necessary accessories and consumables will be delivered (except reagents for immunogold labelling and cleaning).
For the cleaning of glass slides we recommend 1 % DECON 90.

Order number 70 89 01 consists of:

LEICA EM IGL Basic instrument.
Slide carrier blue (30 pcs.)
Slide carrier brown (30 pcs.)
Filter paper (300 pcs.)
Glass slides (100 pcs.)
Grid holder (1 pc.)
Nickel grids (100 pcs.)
Nickel grids carbon/formvar coated, 100 mesh hexagonal (50 pcs.)
Pipette multi 8-channel, 10–100 µl, 1 pc. (96 tips included).
Pipette single channel, 0.5-10 µl, 1 pc. (96 tips included)
Fine forceps with straight tips, non magnetic (1 pc.)
Plastic forceps (1 pc.)
Reagent tub and slide rack for cleaning glass slides
Droplet management pad
Programme protocol pad
Leica EM IGL – PC programming software package

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