

EFFECT OF CX ON CELL PROLIFERATION

Lab 12-08

Folder reference: folder n°1






From: December 01, 2008 - to: December 04, 2008

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|---|-----------|-----------------|-------------|
|  Effect of IL on Cx expression | 01 | December | 2008 |
|  Effect of IL on Cx expression, analyse by Western Blot | 01 | December | 2008 |
|  Cell exposed 18h to IL | 01 | December | 2008 |
| Preparation of cell extract Cos cells, IL, 18h | 01 | December | 2008 |
| Protein separation and immunoblotting Cos cell + IL | 02 | December | 2008 |
| Protein separation and immunoblotting Cos cell + IL sample2 | 02 | December | 2008 |
| WB hybridation Cos cell + IL, Cx | 03 | December | 2008 |
|  New Cx antibody | 04 | December | 2008 |
|  Result of Cell exposed 18h to IL | 04 | December | 2008 |

? Effect of IL on Cx expression

Enclosed by:

- Effect of Cx on cell proliferation

Cells was exposed to various concentrations of IL and the effect on the Cx expression was analysed.

? Effect of IL on Cx expression, analyse by Western Blot

Enclosed by:

- Effect of IL on Cx expression - December 01, 2008

Joined files:

- IL1.pdf

The Cx expression was analysed by western blot

📌 Cell exposed 18h to IL

Enclosed by:

- Effect of IL on Cx expression, analyse by Western Blot - December 01, 2008

For the first experiment, cells were incubated 18h in presence of IL

👤 Preparation of cell extract Cos cells, IL, 18h

Enclosed by:

- Cell exposed 18h to IL - December 01, 2008

Parameters of the manipulation:

| | |
|------------------------|-------------------------------|
| Cell type | Cos cells |
| Cell treatment | IL, 18h |
| Lysis buffer | NP40 / Bridj |
| Volume of lysis buffer | 700µl / condition |
| Sonication | 2x7sec, |
| Incubation | 30min, 4°C |
| Centrifugation | 14 000 rpm, 4°C, 15min |
| Cell pellet | throw out |
| Supernatant | used for western blot |
| DO | kit n° 152345 |

Table of parameters:

| Extract | DO | µg/µl | 75µg protein | Lysis buffer |
|---------|-------|-------|--------------|--------------|
| control | 0.852 | 4.71 | 16 µl | 485 µl |
| + IL | 0.800 | 4.42 | 15 µl | 485µl |

Observations:

Protein separation and immunoblotting Cos cell + IL

Enclosed by:

- Cell exposed 18h to IL - December 01, 2008

Parameters of the manipulation:

| | |
|-------------------------------------|------------------------------------|
| Cell extract | Cos cell + IL |
| Date of preparation of cell extract | 1/12/08 |
| Reduction | Laemmli buffer, 100°C, 5min |
| SDS- Page | 15% |
| Migration | 6mA, ON |
| Electroblotting membrane | PVDF |
| Electroblotting | 3h30, 500mA |
| Colouring | Amido Black |
| Washes | x2 |

Table of parameters:

| | | | | | |
|--------------|----------------|--------------|---------------|----------------|-----------------|
| Control cell | cell + IL(0.1) | cell + IL(1) | cell + IL(10) | cell + IL(100) | cell + IL(1000) |
|--------------|----------------|--------------|---------------|----------------|-----------------|

Observations:

Protein separation and immunoblotting Cos cell + IL sample2

Enclosed by:

- Cell exposed 18h to IL - December 01, 2008

Protocol:

- Immunoblotting%20(CG).pdf

Parameters of the manipulation:

| | |
|-------------------------------------|------------------------------------|
| Cell extract | Cos cell + IL sample2 |
| Date of preparation of cell extract | 1/12/08 |
| Reduction | Laemmli buffer, 100°C, 5min |
| SDS- Page | 15% |
| Migration | 6mA, ON |
| Electroblotting membrane | PVDF |
| Electroblotting | 3h30, 500mA |
| Colouring | Amido Black |
| Washes | x2 |

Observations:

We have changed the protocol

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WB hybridation Cos cell + IL, Cx

Enclosed by:

- Cell exposed 18h to IL - December 01, 2008

Parameters of the manipulation:

| | |
|------------------------------|----------------------------|
| Cell extract | Cos cell + IL |
| Date of membrane preparation | 2/12 |
| Washes | TNN buffer, 3x10min |
| Saturation buffer | TNB, 30min, RT |
| Incubation | 1h, RT |
| Primary antibody | Cx |
| Washes | TNN buffer, 3x10min |
| Saturation buffer | TNB, 30min, RT |
| Secondary antibody | mouse HRP |
| Incubation | 1h, RT |
| Washes | TNN buffer, 3x10min |
| Revelation | ECL, 1 to 3 min |

Observations:

New Cx antibody

Enclosed by:

- Cell exposed 18h to IL - December 01, 2008

For this experiment, I used a new sample of Cx antibody

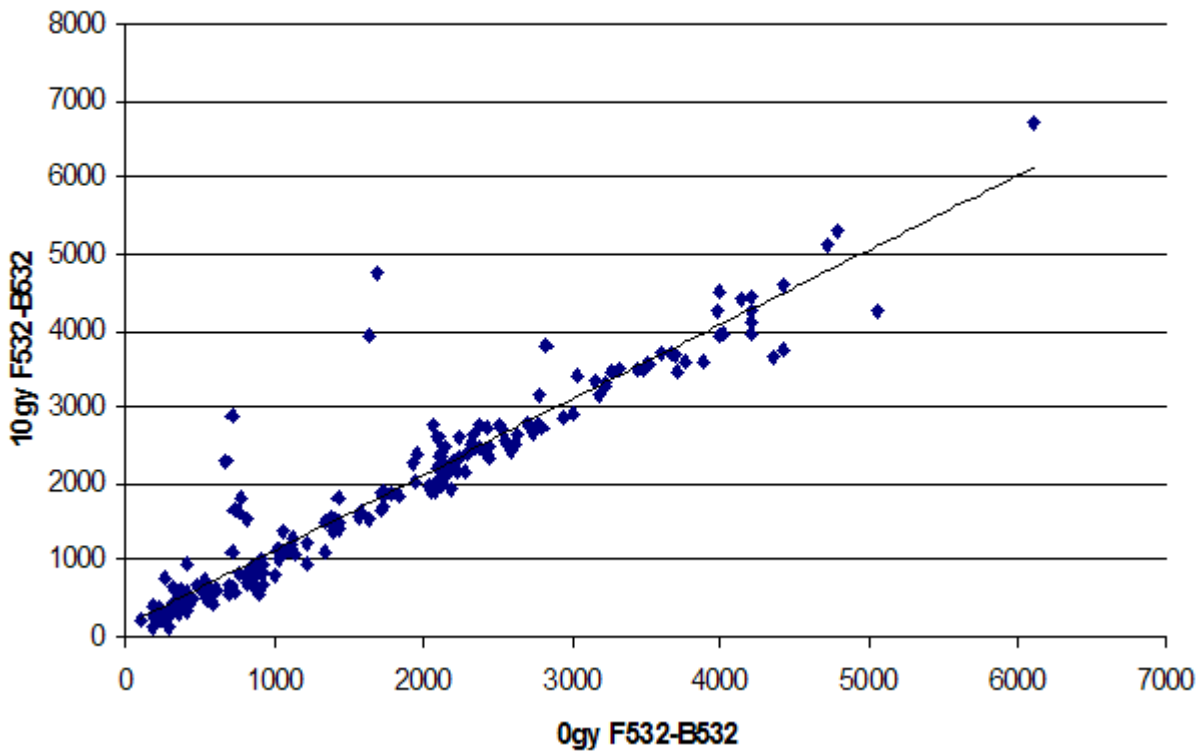
Result of Cell exposed 18h to IL

Enclosed by:

- Cell exposed 18h to IL - December 01, 2008

IL have dose dependent effect of Cx expression.

0gy vs 10gy



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