Interchim Innovations

Interbiotech - BioScience Innovations

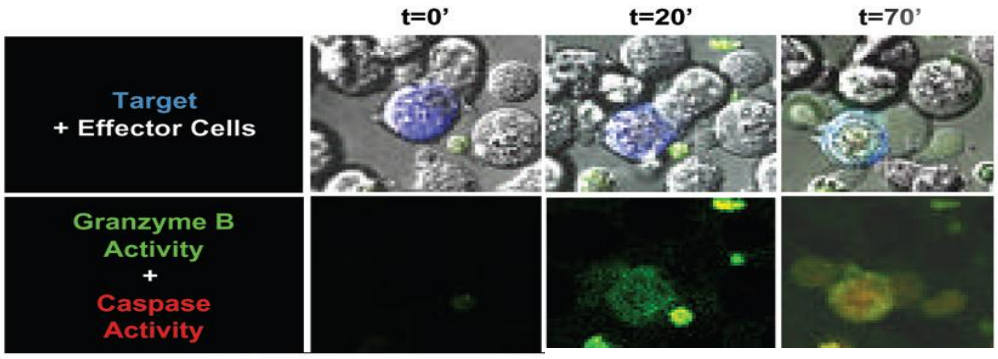
H10E

CTL-Assays: what's new over Chromium^{51} and DHL release assays? how to detect cell-mediated apoptosis, or CD8 cell's memory?

Grantoxilux and Cytoxilux cytotoxicity assays

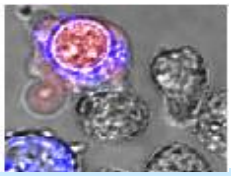
GranToxiLux takes advantage of Granzyme B early event in cell-mediated apotosis, an extremely early event in cytoxicity. **CyToxiLux** takes advantage of Caspase 6 early event in apotosis, an established documented initial activation step in apotosis. Both kits use a fluorescent substrate (for Granzyme B or Caspase6) that results in increased green fluorescence in dying *effector cells*, while the *target cells* are marked by red fluorescence. Following incubation and washing, samples may be analyzed by flow cytometry. Real-time imaging can also be carried out with confocal microscopy. Advantages include:

- **Upstream event & earlier detection**: more informative than chromium Cr^{51} release assay.
- **Quick**: co-incubation of 0.3-2 H (vs. 4 H for 51Cr release assay)
- **Large study period**: hour to days allow long term studies, that is useful for non- or slow-proliferating cells
- **Measure at the cell level**: measured exclusively in target cell, even in mixed populations
- **Compatible with multiparametric FCM and microscopy analysis**: can be combined with immunophenotypic analyses and multiparameter flow cytometry to empowers data exploitation(a)
- **Broadly applicable**: clear solutions, serum, cell suspensions, or on a microscope slide
  No seric interferences: avoid this limitation occuring with LDH and Formazan methods
- **No pre-labeling of cells**: avoid this limitation of 51Cr method



Jurkat Cells
+NK-92 Cells

Caspase+
Jurkat Cell



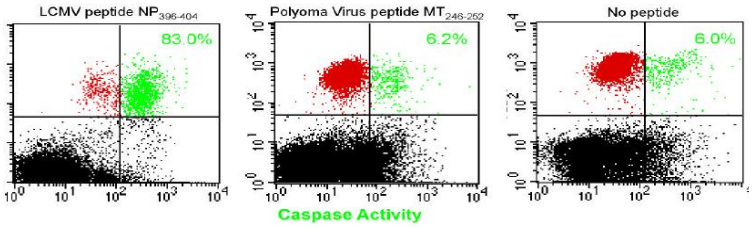
Grantoxilux cytotoxicity assay (Fluo.) Measure the GranzymeB (path of cell-mediated apoptosis), with TFL4 vial (Red Target cell Marker)	**BP8891,80 tests**
Cytoxilux cytotoxicity assay (Fluo.) Measure the caspase-6 (classic path of apoptosis), with TFL4 vial	**BP8881, 80 tests**
Pantoxilux cytotoxicity assay (Fluo.) Measure both the Granzyme B and the caspase-6, with TFL4 vial	**1E3560, 80 tests**
NFL1 vial (Nuclear fluorescent label for eliminating dead cells prior to CTL assay)	**EV1760, 100 tests**

Price and technical sheet on line

GranToxiLux ® , CyToxiLux ® , and PanToxiLux ™

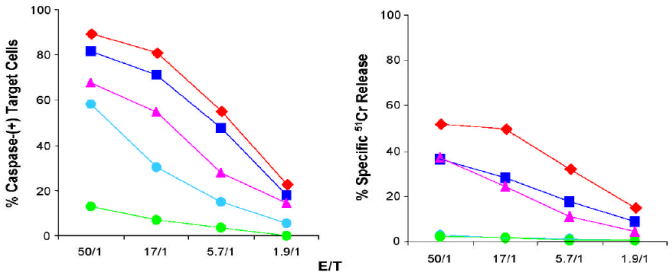
● Applications:

Specific CTL Response Detected by CyToxiLux®



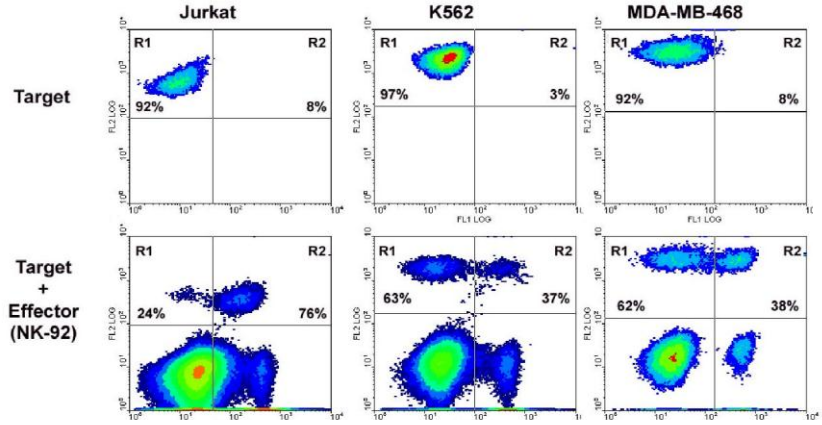
TFL-labeled EL-4 cells were pulsed with either (A) LCMV peptide $NP_{396-404}$, (B) a control polyoma virus peptide $MT_{246-252}$, or (C) no peptide and then cocultured with splenocytes from day 8-post LCMV-infected C57BL/6 mice. OncoImmunin's cell permeable fluorogenic caspase substrate was then added; thirty minutes later cells were washed and subsequently analyzed by flow cytometry. Data show caspase-negative EL-4 cells, caspase-3-positive EL-4 cells, and splenocytes (effectors).

Comparison between CyToxiLux® and ^{51}Cr Release Assays with a Panel of MHC Class I-Restricted Viral Epitopes

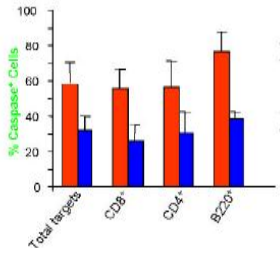


TFL-labeled EL-4 cells were pulsed with LCMV peptides $NP_{396-404}$, GP_{33-42}, $GP_{276-286}$, $NP_{205-212}$ or polyoma virus peptide $MT_{246-254}$. Following coculture with splenocytes from day 8-post LCMV-infected C57BL/6 mice, OncoImmunin's cell permeable fluorogenic caspase substrate was added, cells were washed and subsequently analyzed by flow cytometry.

Comparison of Different Target Cell Killing by NK Cells

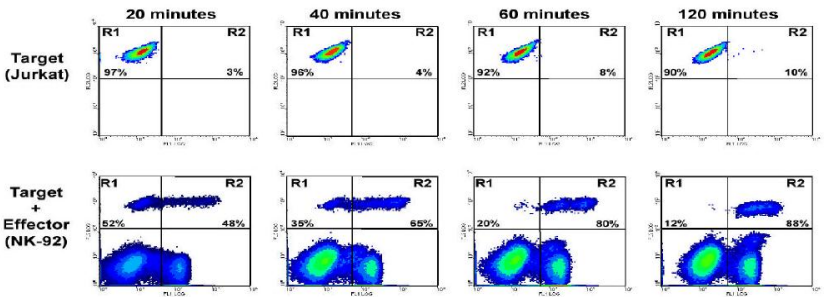


Immunophenotypic Analysis of Cell Subpopulations

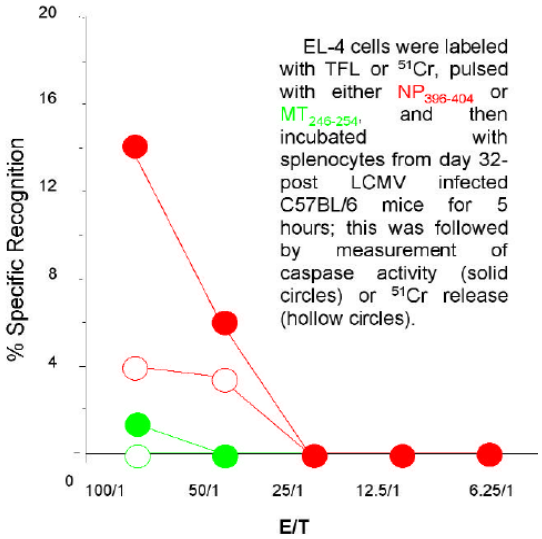


TFL-labeled naïve splenocytes were pulsed with either (A) $NP_{396-404}$ or (B) $MT_{246-252}$ and then cocultured with splenocytes from day 8-post LCMV-infected C57BL/6 mice. Following incubation with OncoImmunin's cell permeable fluorogenic caspase substrate, cells were washed and subsequently stained with anti-CD4, CD8 and B220 mAbs. The percentage of caspase+ cells in each subset is shown.

Time Course of Killing of Jurkat Cells by NK Cells



Direct *Ex Vivo* Memory of CTL Respo



EL-4 cells were labeled with TFL or ^{51}Cr, pulsed with either $NP_{396-404}$ or $MT_{246-254}$, and then incubated with splenocytes from day 32-post LCMV infected C57BL/6 mice for 5 hours; this was followed by measurement of caspase activity (solid circles) or ^{51}Cr release (hollow circles).

●Advantages over other cytotoxicity assays, e.g., Cr^{51} release, LDH release, and PI, include:

(1) cytotoxicity is meas ured as a fundamental bioche mical pathway leading to cell death (cleavage of a cell permeable fluorogenic substrate) rather than merely as the loss of plasma membrane permeability and its sequalae,
(2) sensitivity is enhanced such that relatively weak CTL responses against subdominant epitopes are detectable
(3) rapidity (Effector:Target coincubation ti mes between 0.3 and 2 hours),
(4) measurement of cell death can be carried out exclusively in target cell populations by FCM or fluorescence microscopy,
(5) when combined with immunophenotypic analyses and multiparameter flow cytometr y, cytotoxic lymphocyte-mediated killing of primary host target cells as well as the physiology and fate of effector cells can be directly visualized and monitored

●Differencesn

GranToxiLux ® , CyToxiLux ® , and PanToxiLux ™ are all single cell cytotoxicity assay kits, and can be used for selection of antibodies operating via an antibody-dependent cellular cytotoxicity (ADCC) mechanism in both low and high throughput screening (HTS) modes. They differ by their cell permeable, fluorogenic substrate:
CyToxiLux ® PLUS is designed to detect downstream caspase activity only ,
GranToxiLux ® PLUS is designed to detect downstream granzymeB activity only,
PanToxiLux ™ detects both granzyme B and upstream casp ase activities.

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e-mail interchim@interchim.com - web www.interchim.com

Related products – Cell viability assays

TFL2 vial (Target cell Marker, Fluo, for single 488nm laser)	**FI9080, 100 tests**
TFL4 vial (Target cell Marker, Red Fluo, for dual 488&633nm laser)	**LO221, 100 tests**
MTT (Thialzoyl Blue Tetrazolium Bromide, Ultrapure, CAS: 298-93-1)	**FP-65939A, 1g**
MTT Assay	**FT-45547A, 10x100tests**
Annexin V – FluoProbes488	**FT-BH4140, 500µl / µscopy; FP-BH9390/FCM**
UptiBlue™ Viable Cell Counting Reagent	**UP669412, 25ml** **UP669413, 100ml**

Price and technical sheet on line

Related products – Cell Signalling

Fluo-8 NW Calcium Assay Kit *Medium Removal	**CJ2560, 10 plates***	**CJ2561, 100 plates**
Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium	**CJ2550, 10 plates***	**CJ2550, 100 plates**

* contains Fluo-8 NW, and buffers for performing analysis with 10 plates (96wells, or 384wells)

Fluo-8 – AM	**CP7501, 5x50µg**	**CP7502, 10x50µg**	**CP7504, 1mg**
Fluo-3-AM		**FP-78932A, 1mg**	**FP-78932C, 20x50µg**
Fluo-3-AM FluoroPure grade		**FP-R1245A, 50mg**	
Fluo-3-AM 1% solution in DMSO		**FP-M203A, 1ml**	
BAPTA-AM		**FP-486103, 25mg**	

Price and technical sheet on line

Related products – transfection & siRNA silencing

UptiFectin™-ON DNA Transfection Reagent	**CK5060 114,32 €, 0.5ml** (375 tests*)
UptiFectin™-OFF siRNA Transfection Reagent	**CK5090 105,82 €, 0.5m**

Price and technical sheet on line

Related products – protein expression

LEXSYcon-2 Expression Kit **EGE-1300**

unique protein expression system, that combines advantages of bacteria and mammalian systems (rapid growth, full eukariotic protein folding machinery), and achieve cytosolic or secretory expression using only one vector

Price and technical sheet on line

Related products/documents

Products HighLights Overview

Information inquire

Reply by Fax : +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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