**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 apoptosis, an extremely early event in cytotoxicity.  
*CyToxiLux* takes advantage of Caspase 6 early event in apoptosis, an established documented initial activation step in apoptosis. 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

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**Grantoxilux cytotoxicity assay** (Fluo.)  
BP8891, 80 tests  
Measure the GranzymeB (path of cell-mediated apoptosis), with TFL4 vial (Red Target cell Marker)

**Cytoxilux cytotoxicity assay** (Fluo.)  
BP8881, 80 tests  
Measure the caspase-6 (classic path of apoptosis), with TFL4 vial

**Pantoxilux cytotoxicity assay** (Fluo.)  
1E3560, 80 tests  
Measure both the Granzyme B and the caspase-6, with TFL4 vial

**NFL1 vial** (Nuclear fluorescent label for eliminating dead cells prior to CTL assay)  
EV1760, 100 tests

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*Price and technical sheet on line*
GranToxiLux®, CyToxiLux®, and PanToxiLux™

● Applications:

**Specific CTL Response Detected by CyToxiLux®**

![Image showing specific CTL response detected by CyToxiLux®]

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

![Image showing comparison between CyToxiLux® and 51Cr release assays]

**Comparison of Different Target Cell Killing by NK Cells**

![Image showing comparison of different target cell killing by NK cells]

**Immunophenotypic Analysis of Cell Subpopulations**

![Image showing immunophenotypic analysis of cell subpopulations]

**Direct Ex Vivo Memory of CTL Resp**

![Image showing direct ex vivo memory of CTL response]

- **Advantages** over other cytotoxicity assays, e.g., Cr51 release, LDH release, and PI, include:
  1. Cytotoxicity is measured as a fundamental biochemical pathway leading to cell death (cleavage of a cell permeable fluorogenic substrate) rather than merely as the loss of plasma membrane permeability and its sequelae,
  2. Sensitivity is enhanced such that relatively weak CTL responses against subdominant epitopes are detectable,
  3. Rapidity (Effector:Target coincubation times 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 cytometry, 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.

- **Differences** in 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 granzyme B activity only,
  - PanToxiLux™ detects both granzyme B and upstream caspase activities.
### 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 (Thiazolyl Blue Tetrazolium Bromide, Ultrapure, CAS: 298-93-1) | FP-65939A, 1g |
| MTT Assay | FT-45547A, 10x100 tests |
| Annexin V – FluProbes488 | FT-BH4140, 500µl/µscopy; FP-BH9390/FCM |
| UptiBlue™ Viable Cell Counting Reagent | UP669412, 25ml |
| | UP669413, 100ml |

### Related products – Cell Signalling

| Fluo-8 NW Calcium Assay Kit *Medium Removal* | CJ2560, 10 plates* |
| Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium* | CJ2550, 10 plates* |
| *contains Fluo-8 NW, and buffers for performing analysis with 10 plates (96wells, or 384wells)* |
| Fluo-8 – AM | CP7501, 5x50µg |
| FluorPure grade | CP7502, 10x50µg |
| FluorPure grade | CP7504, 1mg |
| Flu-3-AM | FP-78932A, 1mg |
| Flu-3-AM FluorPure grade | FP-R1245A, 50mg |
| Flu-3-AM % solution in DMSO | FP-M203A, 1ml |
| BAPTA-AM | FP-486103, 25mg |

### 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 €, 0.5ml |

### Related products – protein expression

| LEXSYcon-2 Expression Kit | EGE-1300 |

**LEXSYcon-2 Expression Kit**

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

### Related products/documents

**Products HighLights Overview**

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**Information inquire**

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

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