

Product	Cat.#	Qty	Commentaire
ADHP (yields Resorufin)	FP-39423A	5 mg	Fluorescent HRP substrate
pNPP (ready to use solution)	UP89562D	100 g	Chromogenic HRP substrate
	UP664790	200 ml	
TMB (ready to use solution)	UP15426E	5 g	Chromogenic HRP substrate
	UP664780	1 L	
BCECF-AM	FP-45440C	20 x 50 µg	pH measurment
Calcein-AM	FP-895515	20 x 50 µg	cell tracing, cell viability
CDCFDA-SE	FP-52495A	25 mg	acid pH measurment
Coelenterazine native	UP-972333	1 mg	Reporter Assays, Ca <sup>+</sup> assay, ATP assays
Coelenterazine H	UPR30783	1 mg	Reporter Assays
Coelenterazine CP	UPR30793	1 mg	Reporter Assays
CFDA-SE (CFSE, Green Cell Tracker)	FP-52493A	25 mg	pH measurment, cell tracing
CMTMR (Orange Cell Tracking dye)	FP-12662B	20 x 50 µg	Cell tracer
DiFMUP	58657A		Reporter assay (fluoro substrate)
DiOC6	FP-46764A	100 mg	Mb potential probe
FFP18-AM	FP-AM606B	10 x 50 µg	Near membrane Ca <sup>2+</sup> indicator
FIP18-AM	FPAM608B	10 x 50 µg	Near membrane Ca <sup>2+</sup> indicator
Fluo3-AM	FP-78932C	20 x 50 µg	Ca <sup>2+</sup> indicator
Fluo3FF-AM	FP-AM626B	10 x 50 µg	Ca <sup>2+</sup> indicator
Fluo-4-AM	FP-729712	10 x 50 µg	Ca <sup>2+</sup> indicator
Fluo-4-AM 1mM in DMSO	FP-M2021B	10 x 50 µl	Ca <sup>2+</sup> indicator
FluoProbes®488-NHS	FP-BA6800	1 mg	Green fluorescen label (>FITC)
Fura-2-AM	FP-42776C	20 x 50 mg	Ca <sup>2+</sup> indicator
Fura2FF-AM	FP-AM629B	10 x 50 µg	Ca <sup>2+</sup> indicator
Fura-PE3-AM	FP-AM603B	10 x 50 µg	Ca <sup>2+</sup> indicator
FuraPra-AM	FP-35374C	20 x 50 µg	Mg <sup>2+</sup> indicator
H2DCFDA	FP-467312	100 mg	Oxidative probe
Indo-1-AM	FP-42775A	20 x 50 µg	Ca <sup>2+</sup> indicator
Indo1FF-AM	FP-AM628B	10 x 50 µg	Ca <sup>2+</sup> indicator
IndoPE3-AM	FP-AM602B	10 x 50 µg	Ca <sup>2+</sup> indicator
IPTG	UP84853C	1 g	Reporter assay (inducer)
JC-1 (Depsipher)	FP-52314B	100 test	Mitochondria probe
Luciferin	FP-M1224A	25 mg	Reporter assay (chemil.substrate)
PDF1-AM	FP-86164B	20 x 50 µg	K <sup>+</sup> indicator
Rhod2-AM	FP-661584	20 x 50 µg	Ca <sup>2+</sup> indicator
SBFI-AM	FP-82902B	50 x 20 µg	Na <sup>+</sup> indicator
Synaptracer™1-4 (FM1-43)	FP-51254A	1 mg	Mbr potential indicator (Neurology)
X-Gal	UP40534M	1 g	Reporter assay (chr.substrate)

## Toxicology

Toxicity studies had strong development in recent years in drug development, moving largely from animal testing to in vitro assays. Chromium 51 release assay has become a standard technique, but surprisingly new techniques gained few success. It remains a dominating toxicology assay, although several limitations and drawbacks. Interchim is pleased to provide helpful and innovative alternatives. LDH based assays present very cost effective and fast testing, while GranToxiLux detects earlier toxicity effects and allows you to speed up and combine your testings, especially for cell-mediated cytotoxicity.

### LDH based method

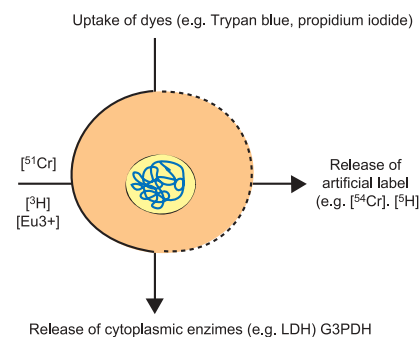
#### DHL™ Cell Cytotoxicity Assay Kit

The damage of cell membrane leads to the release of cytoplasmic enzymes. The measurement of lactate dehydrogenase (LDH) release is a well-accepted assay to estimate cell membrane integrity and quantify cell cytotoxicity. LDH release assay has been proven to correlate very well with the traditional <sup>51</sup>Cr release assay and trypan blue staining method.

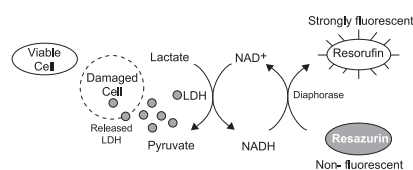
The Cell Cytotoxicity Assay Kit uses resazurin as the fluorogenic indicator to measure the activity of LDH released from damaged cells (Scheme 1). The fluorescent signal is proportional to the number of dead cells (r<sup>2</sup>>0.95), whereas viable cells produce negligible fluorescent signal under the same condition. Consequently, the assay can be performed in a mixture of damaged and viable cells.

This kit is suitable for high throughput screening of the cytotoxicity effect of a variety of compounds.

Description	Cat.#	Qty
DHL™ Cell Cytotoxicity Assay Kit "Fluorimetric"	HT0271	1000 tests
		500 tests (96-well) or 1000 tests (384-well)



#### Methods of cytotoxicity



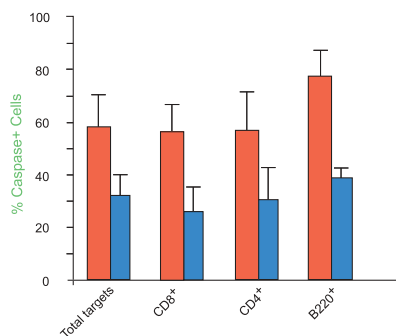
Scheme 1. The enzyme-coupled reaction to detect the activity of released from damaged cells.

The kit contains :  
 Assay mixture  
 Assay buffer  
 Lysis solution  
 Stop solution  
 A detailed protocol

# Drug Discovery : HTS screening & toxicology

## Toxicology

### Immunophenotypic Analysis of Cell Subpopulations



TFL-labeled naïve splenocytes were pulsed with either (A) NP<sub>396-404</sub> or (B) MT<sub>246-254</sub> and then cocultured with splenocytes from day 8-post LCMV-infected C57BL/6 mice. Following incubation with Oncolmin's cell permeable fluorogenic caspase substrate, cells were washed and subsequently stained with anti-CD, CD8 and B220 m220 mAbs. The percentage of caspase+ cells in each subset is shown.

### Granzyme based and Caspase based Toxicology Assays

#### GranToxiLux™ and CyToxiLux® Toxicology assays

Cytotoxicity is measured as functions of fundamental biochemical pathways leading to cell death : in the CyToxiLux® kit, cleavage of a cell permeable fluorogenic caspase substrate, and in the GranToxiLux™ kit, cleavage of a cell permeable substrate for Granzyme B activity in target cells (the Granzyme B is directly from activated effector cells.) . One can measure cell death exclusively in target cell populations by flow cytometry or fluorescence microscopy. These single cell measurements are in contrast to the classic <sup>51</sup>Cr release assay in which bulk quantitation is of an end stage of cell mediated cytotoxicity, *i.e.*, cell lysis.

#### Benefits :

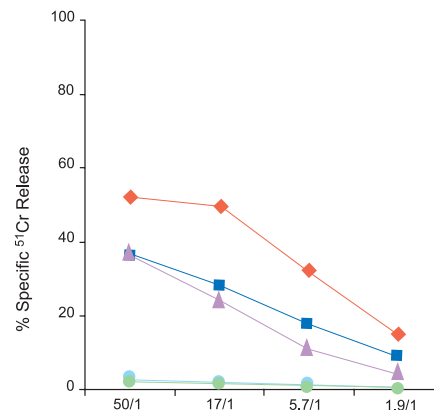
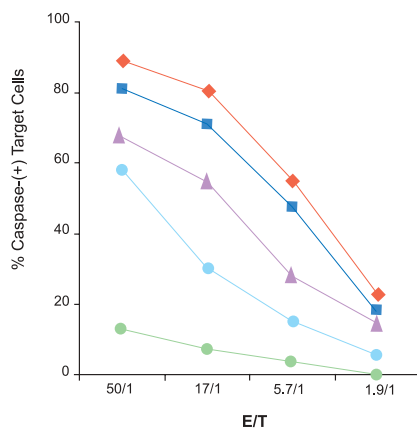
- ◆ More versatile in applications : suits CTL/NK and other factor mediated cytotoxicity, cytotoxicity induced by intracellular agents or xenobiotics, physiology and fate of effector cells
- ◆ More rapid : co-incubation of 0.3-2 H ( vs. 4 H for <sup>51</sup>Cr release assay)
- ◆ More sensitive than the <sup>51</sup>Cr method : can detect relatively weak CTL responses against subdominant epitopes whereas the latter cannot.
- ◆ 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)
- ◆ No seric interferences : avoid this limitation of LDH and Formazan methods
- ◆ No pre-labeling of cells : avoid this limitation of <sup>51</sup>Cr method

Description	Cat.#	Qty
CyToxiLux® kit	BP8881	50 tests*
*the kit contains sufficient reagents for 50 assays in FCM. It may be applied also for microscopy with some modifications.		
Contains :		
Caspase Substrate solution Effector cells		
1 vial Target cell marker for use with single laser instruments (Ar ion(488 nm)		
1 vial Target cell marker for use with dual laser instruments (Ar ion(488 nm) and Red (633 nm))		
Resuspension medium for cell markers vials		
Wash Buffer bottle		
Assay/Culture medium		
GranToxiLux™ kit	BP8891	50 tests*
*the kit contains sufficient reagents for 50 assays in FCM. It may be applied also for microscopy with some modifications.		
Contains :		
3 vials Granzyme B Substrate solution		
1 vial Target cell marker for use with single laser instruments (Ar ion(488 nm)		
1 vial Target cell marker for use with dual laser instruments (Ar ion(488 nm) and Red (633 nm))		
Resuspension medium for cell markers vials		
Wash Buffer bottle		

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#### Comparison between Cy toxiLux® and <sup>51</sup>Cr Release Assays with a Panel of MHC Class I-Restricted Viral Epitopes



TFL- Labeled EL-4 cells were pulsed with LCMV peptides NP396-404, GP33-42, GP276-286, NP 205-212 or polyoma virus peptide MT 246-254. Following coculture with splenocytes from day 8-post LCMV-infected C57BL/6 mice, Oncolmin's cell permeable fluorogenic caspase substrate was added, cells were washed and subsequently analysed by flow cytometry.

### CyToxiLux takes advantage of Caspase 6 early event in apoptosis :

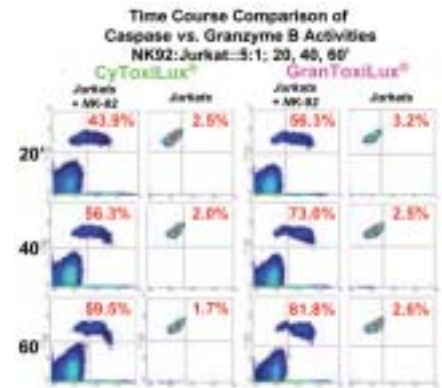
Caspase are established documented initial activation step in apoptosis. CyToxiLux uses a proprietary CaspaLux-6 probe that is very specific of caspase6.

Literature : Nature Med. 8:185-189 (2002)

### GranToxiLux takes advantage of Granzyme B early event in cell-mediated apoptosis:

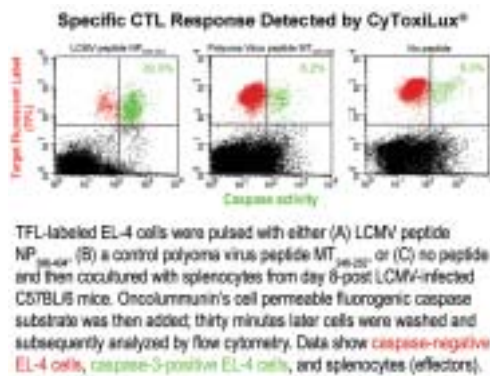
Granzyme B exists in lysosomal granules in an inactive form in Effector cells. When degranulation of Effector cells is induced in the presence of Target cells, Granzyme B as well as other granule contents such as Perforin are taken up by the latter with Granzyme B becoming the first active enzyme inside these Target cells. Thus, measurement of granzyme B activity inside Target cells provides an extremely early quantitative assessment of cell-mediated cellular cytotoxicity.

Literature : Nature Med. 8:185-189 (2002); Methods Mol. Biol. 263:125-140 (2004); J. Immunol. 171:27-31 (2003)



### CyToxiLux and GranToxiLux principle :

Target cells are fluorescently labeled (red) and then coincubated with cytotoxic effector cells in the presence of a fluorogenic granzyme B or Caspase6 substrate. Cleavage of the substrate results in increased green fluorescence in dying cells. Following incubation and washing, samples may be analyzed by flow cytometry. Real-time imaging can also be carried out with confocal microscopy.



### Direct Ex Vivo Memory of CTL Response

