

3.2.3 Summary of methods for studying cytotoxicity

Method/Roche Applied Science product	Label	Parameter analyzed	Assay principle	Advantages	Limitations	For product information, see
[⁵¹Cr] Release Assay⁵⁵	[⁵¹ Cr] prelabel	Damage/leakage of plasma membrane	<ul style="list-style-type: none"> Viable cells are incubated with Na₂[⁵¹Cr]O₄, which binds tightly to most intracellular proteins (prelabeling). After washing, cells are incubated with cytotoxic agent. During this period, labeled proteins are released into the culture supernatant (SN) due to plasma membrane damage. The radioactivity in the SN is determined with a gamma-counter. 	<ul style="list-style-type: none"> Labeling of proteins by [⁵¹Cr] generally independent of the rate of protein synthesis Generally not restricted to target cell type: non-proliferating or slow turn-over populations can be studied Quantitative measurement over a large logarithmic range Measurement of cell death in mixed cell populations; may be used to quantitate cell-mediated cytotoxicity 	<ul style="list-style-type: none"> Radioactive isotope Requires prelabeling and extensive washing of the target cells High spontaneous release: assay limited to cytotoxic events causing high cell lysis over short period of time (2–5 h) For proper intracellular binding [⁵¹Cr]⁶⁺ has to be converted to [⁵¹Cr]³⁺: cells with low metabolic activity may not label sufficiently 	
[³H]-Thymidine ([³H]-TdR) Release Assay⁵⁶	[³ H]-TdR prelabel	Damage/leakage of plasma membrane and DNA fragmentation	<ul style="list-style-type: none"> Cells proliferating <i>in vitro</i> are incubated with [³H] TdR, which is incorporated into the genomic DNA. After they are washed, cells are incubated with a cytotoxic agent. During this period, [³H] labeled DNA is released into the culture SN due to plasma membrane damage. The radioactivity in the SN and in the pellet is determined with a scintillation β-counter. 	<ul style="list-style-type: none"> Quantitative measurement over a large logarithmic range Low spontaneous release: cytotoxic events causing low cell lysis over a prolonged period of time (8–24 h) can be studied Measurement of cell death in mixed cell populations; may be used to quantitate cell-mediated cytotoxicity 	<ul style="list-style-type: none"> Radioactive isotope Requires prelabeling and extensive washing of the target cells Limited to target cells proliferating <i>in vitro</i> 	
DNA Release Assay, nonradioactive Cellular DNA Fragmentation ELISA	BrdU prelabel	Damage/leakage of plasma membrane and DNA fragmentation	<ul style="list-style-type: none"> Cells proliferating <i>in vitro</i> are incubated with BrdU, which is incorporated into the genomic DNA. Cells are incubated with a cytotoxic agent or non-labeled effector cells (for cell-mediated cytotoxicity). During this period, BrdU-labeled DNA is released into the cytoplasm of apoptotic cells or into the culture SN due to plasma membrane leakage of damaged cells. The BrdU-labeled DNA in the SN or cytoplasm is determined with an enzyme linked immunosorbent assay. 	<ul style="list-style-type: none"> Sensitive (10³–10⁴ cells/test required) Labeled cells do not have to be washed Optimal for microplate format Non-radioactive Measurement of cell death in mixed cell populations; may be used to quantitate cell-mediated cytotoxicity Possible to measure apoptosis and necrosis in parallel 	<ul style="list-style-type: none"> Prelabeling of the target cells required Can only assay target cells proliferating <i>in vitro</i> Narrow range of quantitative measurement (only one order of magnitude) 	page 64 of this guide
LDH Release Assay⁵¹ Cytotoxicity Detection Kit, LDH	none	Damage/leakage of plasma membrane	<ul style="list-style-type: none"> Target cells are incubated with cytotoxic agent. During this period, cytoplasmic LDH is released into the culture SN due to plasma membrane damage. The LDH activity in the culture SN is measured by a substrate reaction and quantitated with an ELISA plate reader. 	<ul style="list-style-type: none"> Constitutively expressed ubiquitous protein: assay generally not restricted by target cell type Does not require prelabeling and extensive washing of the target cells 	<ul style="list-style-type: none"> LDH activity in serum: special assay medium (reduced serum concentrations or BSA instead of serum) required Spontaneous release of LDH by target cells and effector cells: assay limited to cytotoxic events causing high cell lysis over short period of time (2–8 h) 	page 61 of this guide
Metabolic activity assays Cell Proliferation Kit 1 (MTT), Kit II (XTT), Reagent (WST-1)	BrdU	Reduced metabolic activity	<ul style="list-style-type: none"> Dye solution is added to cells cultured in MTP and cells are incubated. Viable (metabolically active) cells cleave tetrazolium salts to colored formazan compounds; dead cells do not. Amount of formazan is quantitated with an ELISA plate reader. 	<ul style="list-style-type: none"> No cell type restriction Does not require prelabeling and washing of the cells Optimal for microplate format 	<ul style="list-style-type: none"> Increased metabolic activity of effector cells may mask target cell death during cell-mediated cytotoxicity No changes in the metabolic activity during the early phases of apoptosis 	pages 85–87 of this guide

▲ Table 12: Methods for studying cytotoxicity.

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