abcam

Tools Guide

# Cell viability, cytotoxicity, proliferation, cell cycle, and apoptosis assays guide

Understand your options when you need a cell health assay

The analysis of cell viability, cytotoxicity, cell cycle state, cell proliferation, and cell death are critical to most cell-based studies. Use this guide to understand your options when you need to assay cell health.

Choose the right assay to fit your experimental needs:

- **Cell viability and cytotoxicity assays based on cell metabolism/enzyme activity**
Measure the rate of continuing cellular activities, such as metabolism.

**- Cell viability and cytotoxicity assays based on cytolysis/membrane leakage**
Test for cell membrane damage, either by measuring the leakage of cellular enzymes or staining with membrane-impermeable dyes.

**- Cell proliferation and cell cycle assays**
Monitor the growth of a cell population, detect generations of daughter cells, or analyze the cell cycle state of a cell population. You can use these tests to assay cell viability.

**- Cell death analysis/apoptosis assays**
Measure the markers present in different types of cell death.

Remember: no one method gives a perfect view of cell viability, proliferation, or cell death. It is almost always most effective to combine several different methods.

# Cell viability and cytotoxicity assays: cell metabolism and enzyme activity

You can use several methods to assay ongoing cellular metabolism and enzyme activity to quantify cell viability and cytotoxicity.

Methods include

- Dyes reduced by cellular enzymes
- Mitochondrial membrane potential dependent fluorescent dyes
- Cellular esterase cleaved fluorescent dyes
- ATP and ADP assays
- Assays to measure glycolytic flux and oxygen consumption

See below to learn more, or for the deeper analysis of mitochondria, oxidative stress, metabolites, and metabolic activity, see www.abcam.com/metabolismassays.

## Dye reduction assays

Tetrazolium cell viability assays rely on cellular dehydrogenases to form a colored formazan product, which is measured by absorbance. Other assays use the reduction of resazurin, by electron acceptance from the mitochondrial respiratory chain, to form the fluorescent resorufin.

### Tetrazolium family

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| MTT | Plate reader | Original tetrazolium assay; still very popular. Only tetrazolium assay that needs a wash / solubilization step. | ab211091 |
| MTS | | Most popular assay. More heavily used than WST-1. | ab197010 |
| WST-1 | | More sensitive than MTT, XTT or MTS. | ab155902<br>ab65473<br>ab65475 |
| Cell Counting Kit-8 / CCK-8 / WST-8 | | | |
| XTT assay | | | |

### Resazurin family

Resazurin is equivalent to the active ingredient of ThermoFisher's alamarBlue®.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| Resazurin | plate reader, microscope, flow cytometer | Fluorometric (Ex/Em 535–560/560–615) or colorimetric. No-wash assay. Fluorescent readout enables multiplexing with other assays. | ab129732 |

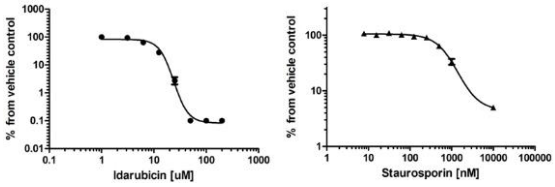


**Figure 2.** Jurkat cells treated with idarubicin (A) or staurosporin (B) were analyzed with Resazurin assay ab129732.

## Mitochondrial membrane potential-dependent dyes

There are several dyes available that accumulate in mitochondria due to the mitochondrial membrane potential and you can use these to identify viable cells. A loss of membrane potential and loss of staining is used to assay for apoptosis.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| TMRE/TMRM | Plate reader, microscope, flow cytometer | Most popular Abcam mitochondrial membrane dye assay. Ex/Em 549/575 nm. Washed out of mitochondria after fixation. | ab113852 |
| JC-1/JC-10 | | JC-1 (Ex/Em 530/530–570) and JC-10 (Ex/Em 590/520–570) form red aggregates at high concentrations (unaggregated dye is green). Loss of membrane potential causes loss of dye and increased green fluorescence. Washed out after fixation. | JC-1: ab113850 JC-10 (more soluble than JC-1): ab112134 ab112133 |
| Mitotracker Red | | Ex/Em 579 /599. Not washed out after fixation. | |
| Rhodamine 123 | | Ex/Em 507/529. Washed out after fixation. | |
| MitoNIR | Plate reader, flow cytometer | Ex/Em 635/660. | ab112149 ab112150 |
| MitoOrange | | Ex/Em 540/590. | ab138898 ab138899 |

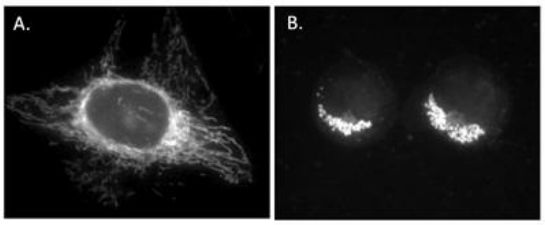


**Figure 3.** Cell staining with TMRE kit ab113852. A: Healthy HeLa cells. B: Healthy Jurkat cells.

## Esterase cleavage

Calcein and similar hydrophobic dyes diffuse into cells and are cleaved by intracellular esterases in live cells. The hydrophilic fluorescent product is retained within the cell.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| Calcein AM | Plate reader, microscope, flow cytometer | Ex/Em 495 / 515 nm | |
| Calcein violet AM | Plate reader, microscope, flow cytometer | Ex/Em 405/460 nm | ab176748 |
| Esterase-cleaved blue | Plate reader | Ex/Em 360/450 nm | ab112120 |
| Esterase-cleaved green | Plate reader, microscope | Ex/Em 490/520 nm | ab112122 |
| Esterase-cleaved near IR | Plate reader | Ex/Em 633/660 nm | ab112123 |

## ATP assays

Most assays use a cell membrane permeabilization agent to release ATP; light is produced using ATP-dependent luciferase. Other ATP assays use the ATP-dependent phosphorylation of glycerol (or other substrates).

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| Luminescence ATP assay | Luminometric plate reader | No-wash assay. | ab113849 |
| Luminescence ADP/ATP assay | | No-wash assay. After ATP analysis, ADP is converted to ATP for detection. | ab65313 |
| ATP phosphorylation assay | Plate reader | No-wash assay used with cell lysates.<br>Not as sensitive as luminescence assays.<br>Fluorometric (Ex/Em 535/587 nm) is more sensitive than colorimetric. | ab83355 |

## Oxygen consumption and glycolysis assays

The rate of oxygen consumption indicates the level of cellular metabolic activity. Analysis of intracellular oxygen levels and glycolysis activity allow deeper investigation.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| Extracellular oxygen consumption | Plate reader | No-wash assay.<br>Dye signal (Ex/Em 380/650 nm) increases as respiration lowers $O_2$ levels.<br>No need for specialized instrument. | ab197243 |
| Intracellular oxygen levels | | Dye fluorescence (Ex/Em 340/642) is quenched by intracellular oxygen. No-wash assay. | ab197245 |
| Glycolysis activity | | No-wash assay.<br>Lactate production causes extracellular acidification and increased dye fluorescence (Ex/Em 340-380/615 nm). | ab197244 |

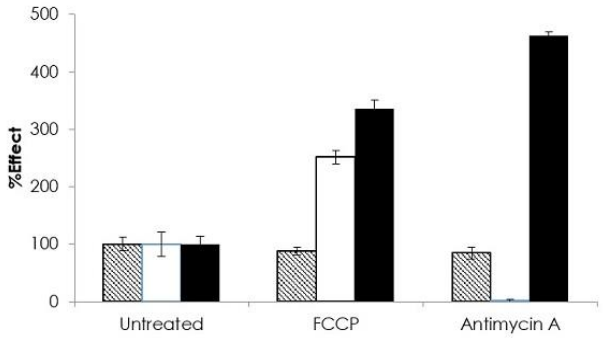


**Figure 4.** HepG2 cells treated with antimycin A and FCCP and tested with assays for ATP (gray, ab113849), oxygen consumption (white, ab197243) and glycolysis (black, ab197244).

# Cell viability and cytotoxicity assays: cytolysis and membrane leakage

You can look at damage to the cell membrane to assay for severe cellular damage or cell death.

Methods include

- Measurement of the activity of enzymes that leak into the extracellular medium
- Membrane impermeable dyes that enter and stain cells upon membrane damage
- Amine-reactive dyes bind weakly to the surface of live cells and create a brighter staining in dead cells

Membrane impermeable dyes are often used, with dyes that stain live cells, in combined live:dead cell assays.

## Enzyme leakage

The most popular enzyme leakage cell viability assay is for lactate dehydrogenase.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| LDH/Lactate dehydrogenase | Plate reader | LDH oxidizes lactate and a colored, or fluorescent (Ex/Em 535/587 nm), product is formed. | ab65393<br>ab197004 |
| AK/Adenylate kinase | | AK converts ADP to ATP with detection via luciferase light-generation.<br>AK activity is not as enduring as LDH. | |

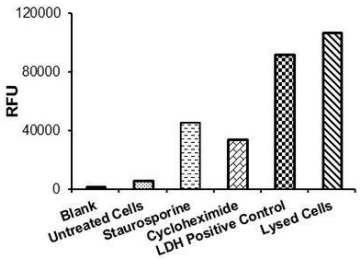


**Figure 5.** LDH assay used with staurosporine or cycloheximide HeLa cells, untreated cells, LDH positive control, and lysed cells.

## Membrane impermeable dyes

Cell viability assays often use membrane-impermeable fluorescent dyes (mostly DNA stains) that stain cells with damaged cell membranes. Propidium iodide has largely been replaced by DRAQ7™ and 7-AAD for cell viability assays due to its broad emission spectra and tendency to bind to live cells.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| DRAQ7™ | Flow cytometer, microscope | Ex/Em 633 & 647/665–800 nm. DNA stain. | ab109202 |
| 7-AAD | | Ex/Em 488/647 nm. DNA stain. | |
| Propidium Iodide | | Ex/Em 536/617 nm. DNA stain.<br>Leaches from cells over time. | ab14083 |
| Ethidium homodimer-1 | | Ex/Em 528/617. DNA stain. | |
| Trypan blue | Microscope | Non-fluorescent cell stain. Classic cell viability assay that requires cell counting. Tedious and prone to manual error. | |

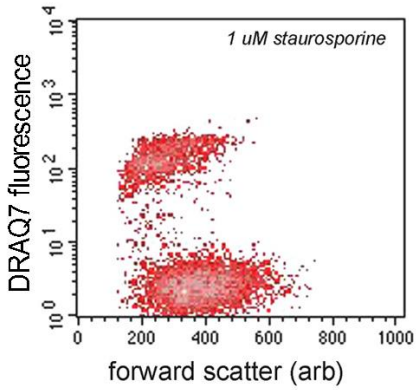


**Figure 6.** Jurkat cells treated with staurosporine to induce cell death show DRAQ7™ staining (top half of the plot).

## Amine-reactive dyes for live:dead cell assays

Amine-reactive dyes weakly stain viable cells by binding to cell surface amines and strongly stain membrane-compromised cells by reacting with intracellular amines. Dead and live cells can be differentiated by fluorescence level.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| Amine-reactive dyes | Flow cytometer | Ex/Em 410/450 nm.<br>Fixation compatible (applies to all dyes in this table). | ab176738 |
| | | Ex/Em 408/512 nm | ab176739 |
| | | Ex/Em 398/550 nm | ab176740 |
| | | Ex/Em 353/442 nm | ab176741 |
| | | Ex/Em 498/521 nm | ab176742 |
| | | Ex/Em 547/573 nm | ab176743 |
| | | Ex/Em 583/603 nm | ab176744 |
| | | Ex/Em 649/660 nm | ab176745 |

## Combined dye live:dead cell assays

Multiple dyes can be combined in a single live:dead cell assay. Examples include the popular Live and dead cell assay (ab115347) with ethidium homodimer to label dead cells and an esterase-cleaved dye for live cells. The alternative, Cell viability assay kit (fluorometric – dual green/red) (ab112121), includes a red DNA staining dye for dead cells and a green esterase-cleaved dye for live cells.

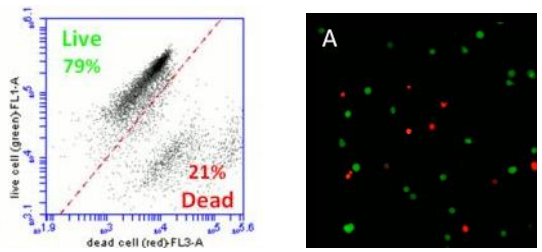


**Figure 7.** Left: Live:dead cell assay (ab115347) used with live (upper left) and dead (lower right) cells. Right: Etoposide treated cells stained with ab115347. Live cells stain green and dead cells are red.

# Cell proliferation and cell cycle assays

These assays monitor the growth rate of a cell population, detect daughter cells in a growing population, or analyze the proportions of cells in different stages of the cell cycle.

Methods include

- DNA staining dyes for cell cycle analysis
- Dye dilution assays
- Incorporation of nucleoside analogs during DNA synthesis

## DNA-staining dyes

DNA-staining dyes are commonly used in flow cytometry to measure the DNA content in cell populations and assay for cell cycle state. Propidium iodide is the mostly commonly used dye.

| Dye | Instrument | Note | Assay kits |
|---|---|---|---|
| Propidium iodide | flow cytometer | Ex/Em 536/617 nm | ab14083, ab139418 |
| Nuclear Green CCS1 | | Ex/Em 490/525 nm | ab112116 |
| Nuclear Red CCS1 | | Ex/Em 490/620 nm | ab112117 |
| DRAQ5™ | | Ex/Em 633&647/665–800 nm | ab108410 |
| DAPI | | Ex/Em 358/461 | |
| Hoechst 33342 | | Ex/Em 350/461 | |
| Hoechst 33258 | | Ex/Em 350/461 | |
| 7-AAD | | Ex/Em 488/647 nm | |

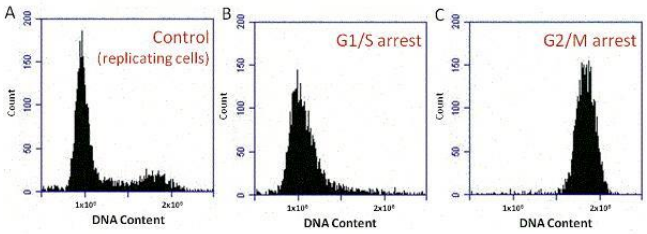


**Figure 8.** Propidium iodide flow cytometry kit (ab139418) used with thymidine (B) and nocodazole (C) treated HeLa cells. Peaks show 2N and 4N DNA content.

## Dye dilution assays

The dyes in dye dilution assays are retained within cells over multiple generations. Daughter cells receive half of the dye of parent cells and assays are analyzed on a flow cytometer. Carboxyfluorescein succinimidyl ester (CFSE) is the longest established dye.

| Assay | Instrument | Notes | Assay kits |
|---|---|---|---|
| CFSE | flow cytometer | Ex/Em 492/517 nm. Cytotoxic at higher concentrations. | ab113853 |
| CytoLabel Blue | flow cytometer, microscope | Ex/Em 403/454 nm | ab176726 |
| CytoLabel Green | | Ex/Em 511/525 nm | ab176735 |
| CytoLabel Red | | Ex/Em 628/643 nm | ab176736 |
| CytoLabel Orange | | Ex/Em 542/556 nm | ab176737 |

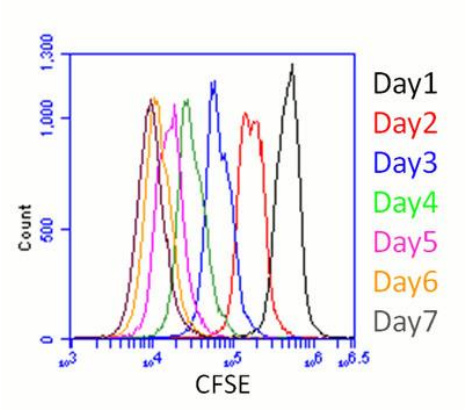


**Figure 9.** Flow cytometry analysis of CFSE (ab113853) dilution assay.

## Nucleoside analogs

Bromodeoxyuridine (BrdU) and ethynyldeoxyuridine (EdU) assays measure the incorporation of BrdU or EdU into newly synthesized DNA during DNA replication. Unlike BrdU, which is detected using antibodies, EdU can be easily directly labeled, either with a fluorescent dye or biotin for colorimetric or fluorometric detection via streptavidin-HRP. Edu staining is consistent with further antibody staining, unlike the harsher BrdU protocol.

| Assay | Instrument | Assay kits |
|---|---|---|
| EdU | Microscope, flow cytometry, plate reader | ab219801<br>ab222421 |
| BrdU | Plate reader, microscope | ELISA: ab126556/ab126572<br>IHC: ab125306 |

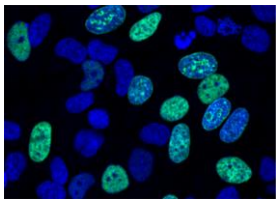

**Figure 10.** EdU staining of proliferating HeLa cells. DNA (blue) was stained with Hoechst 33342 (ab145597). Green cells are EdU + Hoechst-positive.

## Other methods of assaying proliferation

For the analysis of cell proliferation within tissue samples, or sometimes within cell culture, it is common to use antibodies to stain for the presence of Ki67 or PCNA.

Although little used at high throughput, the classical method of assaying cell proliferation is to use a clonogenic/clonogenicity assay. In this assay, cells are plated out at a low density and then the number of colonies formed is counted.

## Senescence assays

The most common marker of senescent cells is the overexpression and accumulation of the endogenous lysosomal beta-galactosidase (SA-beta-gal). Beta-gal activity is detected using a colorimetric or fluorometric substrate.

| Assay | Instrument | Assay kits |
|---|---|---|
| Beta-gal | microscope, plate reader | ab65351 |

## Cell death/apoptosis assays

You can determine how cells are dying by measuring markers that are activated in different types, and at different stages, of cell death.

Methods include

- Annexin V binding of cell surface phosphotidylserine
- DNA condensation and fragmentation (TUNEL) assays
- Caspase activation and detection assays
- Mitochondrial membrane potential dependent dyes
- Cytochrome C release assays
- Glutathione assays
- Assays for necrosis, anoikis and autophagy

For a more detailed examination of cell death, and assays to analyze it, see our three comprehensive guides to apoptosis, necroptosis, and autophagy [hyperlink to guides].

### Annexin V assay

Annexin V binds to phosphatidylserine, which migrates to the outer plasma membrane in apoptosis. Analysis is typically by flow cytometry. Pair Annexin V with a membrane impermeable dye like 7-AAD to distinguish between intact, apoptotic, and necrotic cells (eg see ab214663, ab214484, or ab214485).

| Annexin V conjugate | Ex/Em | Assay kits |
|---|---|---|
| FITC | 495/519 | ab14085, ab14082 |
| Cy3 | 548/561 | ab14142, ab14143 |
| Cy5 | 647/665 | ab14150, ab14147 |
| PE | 496/576 | ab14155, ab14154 |
| PE-Cy5 | 565/693 | ab14159 |
| EGFP | 488/530 | ab14153, ab14152 |
| Biotin | | ab14190, ab14165 |

For a full list of Annexin V dye conjugates, see www.abcam.com/AnnexinV

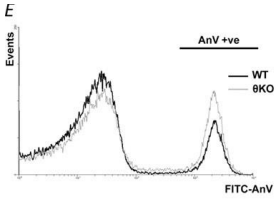


**Figure 11.** Murine platelets were stimulated with CRP in the presence of annexin V-FITC. The annexin V-positive population is indicated (AnV +ve).

### DNA condensation/fragmentation

DNA condensation in apoptosis can be measured using DNA stains to visualize condensed nuclei.

DNA fragmentation can be measured using agarose gels. In the TUNEL assay, the 3' ends of DNA fragments are labeled with deoxyuridine either conjugated to a fluorescent dye or biotin.

| Assay | Instrument | Assay kits |
|---|---|---|
| DNA fragmentation | Gel electrophoresis | ab66090, ab65627, ab66093 |
| TUNEL | Flow cytometry, fluorescence microscope, microscope | ab66108, ab66110, ab206386 |

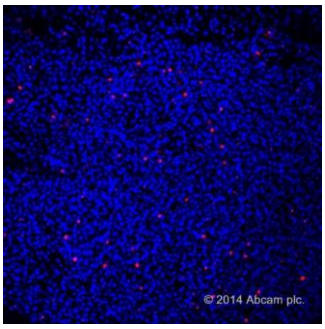


**Figure 12.** TUNEL staining in whole-mount *Hydractinia echinata* using red TUNEL assay kit (ab66110) with DAPI counterstain (blue).

## Active caspase detection

Activated caspases can be detected using antibodies with IHC, western blotting, or flow cytometry.

Caspase activity assays either use peptide substrates, which are cleaved by caspases in cell extracts, or similar substrates that bind to activated caspases in live cells. Caspase specificity varies by substrate.

For information on our assays for caspases 1 through 12, formulated either for cell lysates with analysis by plate reader, or for live cells with analysis by flow cytometer, microscope or plate reader, see www.abcam.com/caspaseassays. For more information about caspase substrates, see www.abcam.com/caspsubguide

We also offer assays for cathepsin and calpain activity analysis: cathepsin D (ab65302), cathepsin B (ab65303), cathepsin L (ab65306), and calpains (ab65308).

## Mitochondrial membrane potential-dependent dyes

Dyes that accumulate in mitochondria due to the mitochondrial membrane potential are also used in the analysis of apoptosis. For more information, see the earlier section on metabolism-based assays. Apoptotic cells stain more weakly with these dyes due to the loss of membrane potential.

## Cytochrome C release

Cytochrome C is released into the cytoplasm following total loss of mitochondrial membrane potential.

| Assay | Instrument | Assay kits |
|---|---|---|
| Cytochrome C | Western blot, fluorescence microscope | ab110415, ab110417, ab65311 |

## Glutathione assay

Glutathione assays are also used for the analysis of apoptosis.

| Assay | Instrument | Assay kits |
|---|---|---|
| GSH/GSSG assay | Fluorometric plate reader | ab138881 |

## Necrosis, anoikis, and autophagy

We offer several kits for studying other forms of cell death: necrosis and apoptosis (ab176749 and ab176750), anoikis (ab211153), and autophagy (ab133075 and ab139484).