

# Cell-based Assays

## Cell-based Assays

The understanding of gene and protein functions and the regulatory mechanisms controlling these, as well as the screening for potential inhibitors or inducers of biological processes, all require the analysis of functional cells. Cultured cells constitute a comprehensive unit that enables functional analysis. Screens in cell-based systems offer accurate representation of the real-life cell model and the possibility of a dynamic experiment through *in vitro* monitoring of the numbers, phenotype or behavior of the live cells.

The diversity of biomolecules present in cells and the need to characterize their presence in normal development/diseased states or treated/untreated conditions leads to an increased demand for corresponding assays.

In addition to a broad collection of fluorescent proteins ranging in color from blue to far-red, a wide variety of cell-based assays designed for greater accuracy and increased throughput is provided. They allow for the analysis of apoptosis, cell proliferation and cell damage as well as cell adhesion, migration and invasion. They provide fully quantitative results with no manual cell counting required. The majority of the assays is performed in microplate format. Analysis is carried out by colorimetric or fluorometric detection.

[www.biocat.com/cell-based](http://www.biocat.com/cell-based)

*In this brochure, new and especially important products are highlighted. For the complete listing as well as for more detailed product and order information, please use the respective web link at the end of each section.*

**Apoptosis**

**Caspases & Caspase Inhibitors**

**Cytotoxicity & Proliferation**

**Cell Adhesion**

**Cell Migration & Invasion**

**Fluorescent Proteins**

# Apoptosis

- Analyze different stages of apoptosis
- Differentiate between apoptotic and necrotic cells
- Select the assay suitable for your sample type

Apoptosis, or programmed cell death, plays an important role in many physiological and diseased conditions. In contrast to necrotic cells, apoptotic cells are characterized morphologically by compaction of the nuclear chromatin, shrinkage of the cytoplasm and production of membrane-bound apoptotic bodies. Biochemically, apoptosis is distinguished by fragmentation of the genome and cleavage or degradation of several cellular proteins.

As no single parameter fully defines cell death in all systems, it is often advantageous to use several different approaches when studying apoptosis. A large number of assays is offered for the detection of apoptosis at the early, middle and late stages of the apoptotic cascade, and for the analysis of apoptotic events that occur in different areas of the cell, including the plasma membrane, cytoplasm, mitochondria and nucleus.

Sample Type	Product
Live Cells (Suspension or Adherent)	Annexin V Apoptosis Assay Kits CaspGlow Caspase Assay Kits MitoCapture Apoptosis Assay Kit
Cell Lysate (Fresh or Frozen)	Caspase (1-12) Fluorometric Assay Kits Caspase (1-10) Colorimetric Assay Kits
Tissue Lysate (Fresh or Frozen)	Caspase (1-12) Fluorometric Assay Kits ADP, ATP & ADP/ATP Ratio Assay Kits
Tissue Sections (Frozen or Paraffin)	Apo-BrdU-IHC Active Caspase-3 Antibody
Cell Area	Product
Mitochondrial Changes	MitoCapture Apoptosis Assay Kit Cytochrome c Releasing Apoptosis Assay Kit
Membrane Changes	Annexin V Assay Kits
Nuclear Changes	DNA Ladder Assay Kits TUNEL Assay Kits
Cytosol Changes	Caspase Assay Kits
Purpose	Product
Measure Cytochrome c Release	Cytochrome c Releasing Apoptosis Assay Kit
Screen Caspase Inhibitors	Caspase Inhibitor Screening Kits
Block Apoptotic Gene Expression	siRNA Apoptotic Gene Expression Vectors
Study Apoptosis-related Non-Caspase Proteases	Calpain Assay Kit Cathepsin Assay Kits
Isolate Apoptotic Cells	Apoptotic Cell Isolation Kit
Induce Apoptosis	Ready-to-use Apoptosis Inducers
Differentiate between Apoptosis & Necrosis	Annexin V-FITC Apoptosis Kit Annexin V-EGFP Apoptosis Kit

Autophagy is closely associated with apoptosis. Cells use autophagy to digest damaged or defective molecules, effectively eliminating the stressors like misfolded proteins and damaged organelles that can threaten cell health. An extensive collection of autophagy antibodies, including APG8 (MAPLC3), LAMP and APG1, is offered.

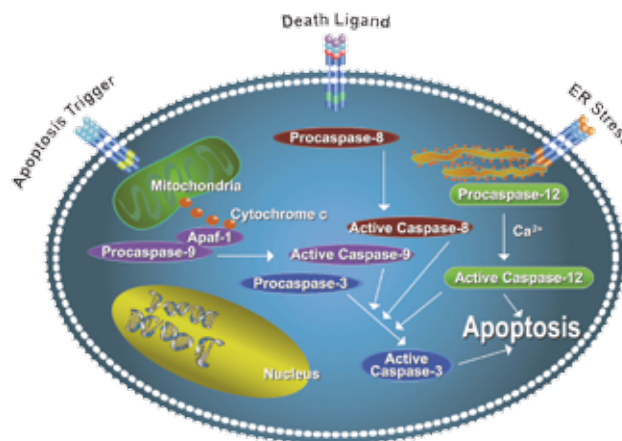
[www.biocat.com/apoptosis](http://www.biocat.com/apoptosis)

# Caspases & Caspase Inhibitors

- Active recombinant caspases and procaspases
- Ready-to-use cell-permeable caspase inhibitors
- High quality and high activity

Caspases are responsible for the deliberate disassembly of the cell into apoptotic bodies during apoptosis. They are present within the cell as inactive proenzymes that are activated by proteolytic cleavage. Caspase-8, -9, -12 and -3 are situated at pivotal junctions in apoptosis pathways.

Caspase-8 initiates disassembly in response to extracellular apoptosis-inducing ligands and is activated in a complex associated with the cytoplasmic death domain of many cell surface receptors for the ligands. Caspase-9 activates disassembly in response to agents or insults that trigger the release of cytochrome c from mitochondria and is activated when complexed with apoptotic protease activating factor 1 (APAF-1) and extra-mitochondrial cytochrome c. Caspase-12 appears to be activated by ER stress. All three caspase pathways lead to caspase-3 activation. Caspase-3 amplifies caspase-8, caspase-9 and caspase-12 initiation signals into full-fledged commitment to disassembly. Caspase-8 and caspase-9 activate caspase-3 by proteolytic cleavage and caspase-3 then cleaves vital cellular proteins or other caspases.



**Caspase activation pathways.**

Active caspases are useful to study enzyme regulation and kinetics, screen caspase inhibitors, determine target substrates and as positive controls in caspase activity assays and Western Blot analysis. High-quality human, mouse and rat recombinant active caspases are provided. Procaspase-3, -7 and -9 are also available for studying caspase activation pathways. Cell-permeable and nontoxic caspase inhibitors irreversibly bind to activated caspases to block apoptosis. Ready-to-use individual caspase inhibitors or caspase inhibitor sets are offered, providing more convenience when studying multiple apoptosis-inhibiting processes.

[www.biocat.com/caspases](http://www.biocat.com/caspases)

# Cytotoxicity & Proliferation

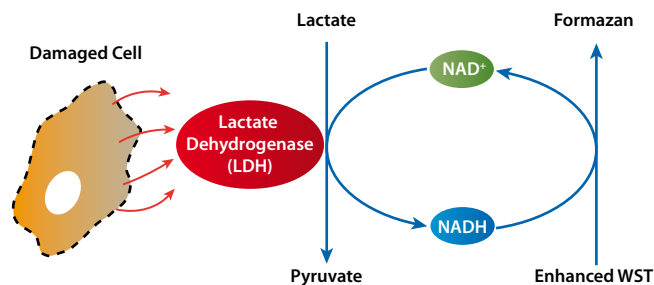
## LDH Cytotoxicity Assay Kit II

- Quantify released LDH with high sensitivity
- Fast and convenient assay based on enhanced WST
- 96-well format, colorimetric detection

Cell death or cytotoxicity is classically evaluated by the quantification of plasma membrane damage. Lactate dehydrogenase (LDH) is a stable enzyme, present in all cell types and rapidly released into the cell culture medium upon damage of the plasma membrane. LDH, therefore, is the most widely used marker in cytotoxicity studies.

The LDH Cytotoxicity Assay Kit II developed by BioVision utilizes enhanced WST reagent for the fast and highly sensitive detection of LDH released from damaged cells: LDH oxidizes lactate to generate NADH, which then reduces enhanced WST to generate yellow colored formazan. The intensity of the color detected at 450 nm correlates directly with the number of cells lysed.

Since enhanced WST is brighter, less amount of culture medium is required for the assay, and thus the background from serum and culture medium is significantly reduced. Using the assay, cells can be cultured in regular 10% serum containing medium, no reducing serum or special medium is required for the assay. In addition, as enhanced WST is more stable, the reaction can be read multiple times, and can also be stopped at any time point during the reaction.



Principle of the LDH Cytotoxicity Assay Kit II.

The **Quick Cell Proliferation Assay Kit II** (colorimetric) is designed to determine the number of living cells. It is based on the cleavage of the tetrazolium salt WST (enhanced version) to formazan by cellular mitochondrial dehydrogenase. The amount of dye generated by the activity of dehydrogenase is directly proportional to the number of living cells.

[www.biocat.com/cytotoxicity](http://www.biocat.com/cytotoxicity)

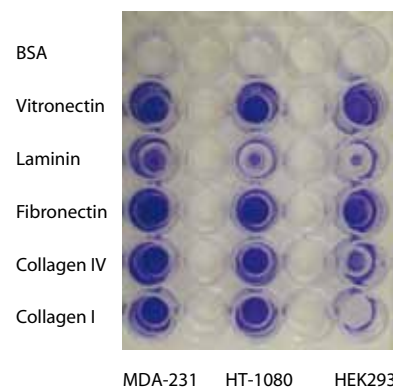
# Cell Adhesion

## CytoSelect Cell Adhesion Assay (ECM Array)

- Quantify cell adhesion without manual cell counting
- Analyze cell interactions with 5 ECM proteins
- Colorimetric or fluorometric detection

Cell adhesion is a complex mechanism involved in a variety of processes including migration & invasion, embryogenesis, wound healing and tissue remodeling. To perform these processes, cells adhere to extracellular matrix (ECM) components (via adhesion receptors), forming complexes with components of the cytoskeleton that ultimately affect cell motility, differentiation, proliferation and survival.

The Cell Biolabs CytoSelect Cell Adhesion Assay (ECM Array) provides a rapid, quantitative method for evaluating the interactions of cells with 5 different ECM proteins, collagen I, collagen IV, fibrinogen, fibronectin and laminin I, using a microplate reader. No manual cell counting is necessary.



### Cell adhesion analysis of different cell lines using the CytoSelect 48-well Cell Adhesion Assay (ECM Array, colorimetric).

Serum-starved cells from three different cell lines were allowed to attach to the ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were stained according to the assay protocol. BSA = Bovine Serum Albumin.

Colorimetric and fluorometric cell adhesion assays utilizing one particular ECM protein are also available.

The **CytoSelect Endothelium Adhesion Assays** provide a robust system for the quantitative determination of interactions between leukocytes or tumor cells and endothelium. Endothelial cells are cultured and form a monolayer, which is treated with the desired activator or inhibitor. Meanwhile, leukocytes/tumor cells are labeled with LeukoTracker/CytoTracker solution, pelleted, washed and resuspended. Cells are added to the monolayer and incubated. Adherent cells are washed, lysed and quantitated by a fluorometer. The kits contain sufficient reagents for the evaluation of 100 assays in a 96-well plate.

In addition, a kit enabling the quantitative determination of leukocyte-epithelium interactions is provided.

[www.biocat.com/adhesion](http://www.biocat.com/adhesion)

# Cell Migration & Invasion

Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis and arthritis.

The initial response of a cell to a migration-promoting agent is to polarize and extend protrusions in the direction of the attractant; these protrusions can consist of large, broad lamellipodia or spike-like filopodia. In either case, these protrusions are driven by actin polymerization and can be stabilized by extracellular matrix (ECM) adhesion or cell-cell interactions (via transmembrane receptors). There are various types of cell migration including chemotaxis, haptotaxis and transmigration.

Cell invasion is related to, and encompasses, cell migration, except that cells do more than migrate. Invasive cells move through the extracellular matrix into neighboring tissues in a process that involves ECM degradation and proteolysis. Metastatic cells produce many proteolytic enzymes (e.g. lysosomal hydrolysates, collagenases, plasminogen activators) while the expression of certain cell surface protease receptors is also increased. The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers.

Cell migration assays developed by Cell Biolabs are offered in two formats: Boyden Chamber Assays and Gap Closure Assays.

	Boyden Chamber Assays	2D Migration Gap Closure Assays
<b>Analysis</b>	Quantitative	Qualitative or Quantitative
<b>Detection Time</b>	Endpoint	Endpoint or Real Time
<b>Detection Method</b>	Plate Reader	Microscopy
<b>Cell Compatibility</b>	Choose membrane pore size to match cell type	Any
<b>Chemoattractant Gradient</b>	Yes	No
<b>Sensitivity</b>	Fair	Good
<b>Adaptability to Automation</b>	Poor	Good
<b>Most Suitable Application</b>	Measure effect of chemoattractant on migration rates	Measure differences in migration rates between treated and untreated cells

[www.biocat.com/migration](http://www.biocat.com/migration)

# Boyden Chamber Assays

Boyden Chamber Assays consist of a cylindrical cell culture insert nested in the well of a cell culture plate. The insert contains a polycarbonate membrane at the bottom with a defined pore size. Cells are seeded into the insert, and migratory cells move through the pores toward the chemoattractant below and can be stained or quantified in a plate reader. Invasive cells may be similarly measured by the placement of a coating of extracellular matrix proteins on top of the membrane.

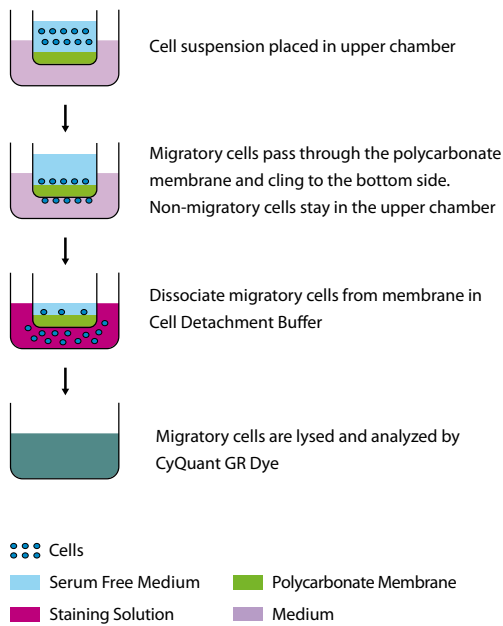
	Cell Types	Pore Size	Insert Coating	Formats
<b>Chemotaxis</b>	Leukocytes Neutrophils	3 µm	None	24-Well 96-Well
	Lymphocytes Macrophages Monocytes	5 µm	None	24-Well 96-Well
	Endothelial Cells Epithelial Cells Fibroblasts Tumor Cells	8 µm	None	24-Well 96-Well
	Astrocytes Slow-moving Cells	12 µm	None	24-Well
<b>Haptotaxis</b>	Endothelial Cells Epithelial Cells Fibroblasts	8 µm	Collagen I (bottom)	24-Well
			Fibronectin (bottom)	24-Well
<b>Transmigration</b>	Leukocytes	3 µm	None	24-Well
	Tumor Cells	8 µm	None	24-Well
<b>Invasion</b>	Endothelial Cells Epithelial Cells Fibroblasts Tumor Cells	8 µm	ECM Matrix (top)	24-Well 96-Well
			Collagen I (top)	24-Well 96-Well
			Laminin I (top)	24-Well 96-Well

## CytoSelect Cell Migration Assays (Chemotaxis)

- Quantify chemotaxis with no manual cell counting
- Measure chemotaxis in less than 6 hours with most cell types
- Uncoated membrane inserts for use with any chemoattractant
- Colorimetric or fluorometric detection

Chemotaxis describes cell migration based on chemicals in a cell's surrounding environment. Chemotaxis can indicate cell migration either toward or away from a particular chemical signal. Cell chemotaxis plays a pivotal role in the progression of cancer and other diseases.

The CytoSelect Cell Migration Assays are ideal for determining the chemotactic properties of cells. The kits utilize polycarbonate membrane inserts in either 24-well or 96-well plates. Membranes are available in 4 pore sizes to accommodate a variety of cell types and sizes, see table above. The membrane serves as a barrier to discriminate migratory cells from non-migratory cells. Migratory cells are able to extend protrusions towards chemoattractants (via actin cytoskeleton reorganization) and ultimately pass through the pores of the polycarbonate membrane. These migratory cells are then dissociated from the membrane and subsequently detected and quantified.



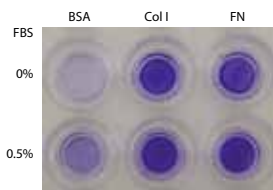
**Principle of the CytoSelect Cell Migration Assays.**

[www.biocat.com/chemotaxis](http://www.biocat.com/chemotaxis)

## CytoSelect Haptotaxis Assays

- Quantify haptotaxis with no manual cell counting
- Measure haptotaxis in less than 6 hours with most cell types
- Collagen I- or fibronectin-coated membrane inserts
- Colorimetric or fluorometric detection

Cell haptotaxis describes cell migration toward or along a gradient of chemoattractants or adhesion sites in the extracellular matrix. The CytoSelect Haptotaxis Assays are optimized for determining the migratory properties of cells. They utilize polycarbonate membrane inserts, 8  $\mu\text{m}$  pore size, ideal for epithelial cells and fibroblasts.



### CytoSelect 24-Well Cell Haptotaxis Assay.

MDA-231 cells were seeded at 150,000 cells/well and allowed to migrate toward FBS for 4 hours. Migratory cells were stained according to the assay protocol.

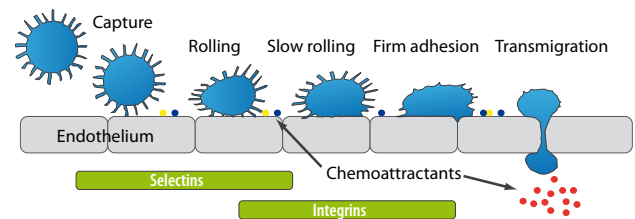
The bottom side of the Boyden chamber insert is coated with collagen I or fibronectin. The membrane serves as a barrier to distinguish between migratory and non-migratory cells. Migratory cells are able to extend protrusions towards the gradient of extracellular matrix density (via actin cytoskeleton reorganization) and ultimately pass through the pores of the polycarbonate membrane. Finally, the non-migrating cells are removed and the migratory cells are stained and quantified.

[www.biocat.com/haptotaxis](http://www.biocat.com/haptotaxis)

## CytoSelect Transmigration Assays

- Quantify cell transmigration with no manual cell counting
- Determine leukocyte/tumor endothelium interactions
- Highly sensitive results on a fluorescence plate reader

Transmigration describes the migration of cells (usually leukocytes or tumor cells) through the vascular endothelium toward a chemoattractant. The initial arrest and attachment of tumor cells to vascular endothelium precedes their extravasation from the blood stream and is a crucial step in the tumor metastatic cascade. Tumor cell extravasation is equivalent, in many respects, to the entry of leukocytes into inflammatory tissue. Leukocyte extravasation consists of multiple, consecutive processes including the capture of circulating leukocytes, subsequent leukocyte rolling, arrest, firm adhesion and transmigration.



**The leukocyte adhesion / transmigration cascade.**

The CytoSelect Transmigration Assays are Boyden chamber-based assays in which the cells transmigrate from the upper chamber (membrane inserts with 3 or 8  $\mu\text{m}$  pore size) through an endothelial monolayer into the lower chamber. Migratory leukocytes/cancer cells may be quantified on a fluorescence plate reader.

[www.biocat.com/transmigration](http://www.biocat.com/transmigration)

## CytoSelect Cell Invasion Assays

- Quantify cell invasion with no manual cell counting
- Plates precoated with ECM gel, collagen I or laminin I
- Colorimetric or fluorometric detection

The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Metastatic cells produce many proteolytic enzymes (e.g. lysosomal hydrolyses, collagenases, plasminogen activators) while the expression of certain cell surface protease receptors is also increased.

The CytoSelect Cell Invasion Assay Kits utilize basement membrane-coated inserts to assay the invasive properties of tumor cells. The upper surface of the insert membrane is coated with a uniform layer of either ECM (extracellular matrix) gel (basement membrane), collagen I or laminin I. Invasive cells are able to degrade the matrix proteins in the layer and ultimately pass through the pores of the polycarbonate membrane. Finally, the cells are removed from the top of the membrane and the invaded cells are stained and quantified.

[www.biocat.com/invasion](http://www.biocat.com/invasion)

## Gap Closure Assays

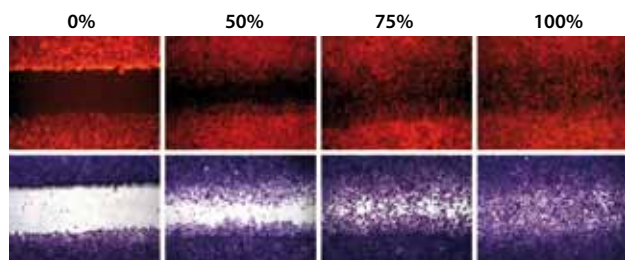
Gap Closure Assays create a defined area across which migratory cells can move. Migration can be monitored in real-time by microscopy. These assays include the new Radius technology which uses a biocompatible hydrogel to create a circular area across which cells can migrate, and the Wound Healing Assay which is a more consistent alternative to the traditional scratch assay.

### CytoSelect Wound Healing Assays

- Measure cell migration, cell proliferation and wound closure
- More consistent compared to homemade scratch assays
- Inert inserts leave no residues that could impede proliferation or migration of cells

Wounded tissue initiates a complex and structured series of events in order to repair the damaged region. These events may include increased vascularization by angiogenic factors, an increase in cell proliferation and extracellular matrix deposition, and infiltration by inflammatory immune cells as part of the process to destroy necrotic tissue. The wound healing process begins as cells polarize toward the wound, initiate protrusion, migrate and close the wound area.

Traditionally scratch assays have been used to study cell migration, cell proliferation and wound healing. However, these assays lack a consistently defined wound gap and can result in high inter-sample variation.



#### Percent closure of MEF/STO cells.

STO cells were tested using the CytoSelect 24-Well Wound Healing Assay. Cells were cultured 24 hours until a monolayer formed, at which time the inserts were removed to begin the assay. Cells were monitored under DAPI labeling and cell staining for determining percent closure.

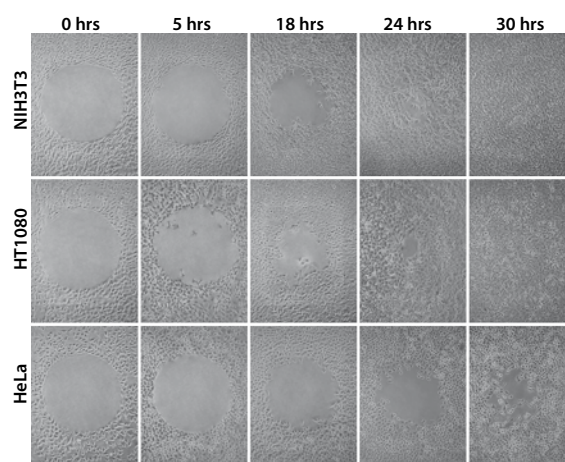
The CytoSelect 24-Well Wound Healing Assay provides a much more consistent method to measure cell migration across a wound field gap *in vitro*. Proprietary inserts generate a consistent wound gap between the cells. Cells are cultured until they form a monolayer around the insert. The insert is removed, leaving a precise 0.9 mm open "wound field" between the cells. Cells can be treated and monitored at this point for migration and proliferation into the wound field. Progression of these events can be measured by imaging samples fixed at specific time points or time-lapse microscopy.

[www.biocat.com/wound\\_healing](http://www.biocat.com/wound_healing)

## Radius Cell Migration Assays

- Defined circular area for precise cell migration measurement
- Qualitative, quantitative, endpoint or real-time analysis
- Compatible with all cell stains, dyes and labels
- Suitable for automation

The Radius Cell Migration Assays provide a unique alternative to conventional cell migration assays using the Boyden chamber. Unlike Boyden chamber assays which may only be analyzed at endpoint, the Radius assay uses a proprietary cell culture plate containing a carefully-defined biocompatible hydrogel (Radius Gel) spot (0.68 mm) centralized at the bottom of each well. When cells are seeded in the well, they will attach everywhere except on the Radius Gel, creating a cell-free zone. Following cell seeding the Radius Gel is removed, allowing migratory cells to move across the area and close the gap.



#### Cell migration time course.

HeLa, HT1080 and NIH3T3 cells were seeded at 100,000 cells/well overnight. After removal of Radius Gel, cells were allowed to migrate for the various times shown.

This format provides a robust *in vitro* system to measure 2-D cell migration, screen potential inhibitors, study cytoskeleton reorganization events or investigate wound healing. It is adaptable to liquid handling equipment and HCS instrumentation.

The Radius Cell Migration Assays are available in 24-, 96- and 384-well plate format. They are also provided with 24-well plates precoated with collagen I, fibronectin or laminin to help promote cell adhesion.

[www.biocat.com/radius](http://www.biocat.com/radius)

# Fluorescent Proteins

## TurboFPs

- Superbright fluorescence
- Very fast maturation
- Optimal for cell labeling

TurboFPs are fluorescent proteins of different colors characterized by superbright fluorescence and superior fast maturation. These proteins are recommended for applications requiring fast appearance of bright fluorescence, including cell and organelle labeling or tracking promoter activity. The far-red marker TurboFP635 is ideal for whole body imaging applications.



**DsRed-Express and TurboFP635 expression in transgenic *Xenopus laevis*.**

Transgenic 2.5 months living animals expressing DsRed-Express or TurboFP635 under the control of cardiac actin promoter are shown from the dorsal side. TurboFP635 (on the right) is excellently visible in the whole body, while DsRed-Express (on the left) can be hardly visualized.

The comprehensive fluorescent protein offering developed by Evrogen comprises expression/source vectors, antibodies, recombinant proteins and stable cell lines.

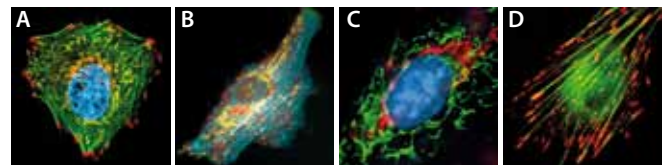
[www.biocat.com/fluorescent\\_proteins](http://www.biocat.com/fluorescent_proteins)

## TagFPs

- Bright monomeric proteins
- Excellent performance in fusions
- Wide color palette

TagFPs comprise monomeric fluorescent proteins that are optimized for protein localization, protein interaction studies and stable expression in long term cultures. They can also be used for other cell labeling applications.

TagFPs demonstrate successful performance in fusions with cellular proteins and can be expressed in various heterologous systems. Ranging in color from blue to far-red, they provide unique possibilities for multicolor labeling of subcellular structures.



**Multicolor labeling of subcellular structures in transiently transfected mammalian cells using TagFPs.**

(A) TagBFP-histone H2B fusion (blue), TagGFP-actin fusion (green), mitochondria-targeted PhiYFP (yellow), Golgi-targeted TagRFP (orange), mKate2-zyxin fusion (red). (B) TagCFP-actin fusion (cyan), mitochondria-targeted PhiYFP (yellow) and mKate2-clathrin fusion (red) in HeLa cells. (C) TagRFP-cytokeratin 14 fusion (red) and mitochondria-targeted TagGFP2 (green) in REF3 cells with Hoechst staining (blue). (D) TagGFP2-actin fusion (green) and mKate2-zyxin fusion (red) in REF52 cells.

	TURBO FLUORESCENT PROTEINS (TurboFPs)					
	TurboGFP	TurboYFP	TurboRFP	TurboFP602	TurboFP635	TurboFP650
Fluorescence color	green	yellow	red (orange)	true-red	far-red	near-infrared
Excitation max (nm)	482	525	553	574	588	592
Emission max (nm)	502	538	574	602	635	650
Quantum yield	0.53	0.53	0.67	0.35	0.34	0.24
Extinction coefficient (M <sup>-1</sup> cm <sup>-1</sup> )	70 000	105 000	92 000	74 400	65 000	65 000
Brightness*	37.1	55.7	61.6	26.0	22.1	15.6
Brightness, % of EGFP	112	169	187	79	67	47
pKa	5.2	5.9	4.4	4.7	5.5	5.7
Structure	dimer	dimer	dimer	dimer	dimer	dimer
Cell toxicity	not observed	at high concentrations**	not observed	not observed	not observed	not observed
Aggregation	no	at high concentrations**	no	no	no	no
Maturation rate at 37°C	super fast	super fast	super fast	fast	super fast	super fast
Molecular weight (kDa)	26	26	26	26	26	26

	TAG FLUORESCENT PROTEINS (TagFPs)					
	TagBFP	TagCFP	TagGFP2	TagYFP	TagRFP	mKate2
Fluorescence color	blue	cyan	green	yellow	red (orange)	far-red
Excitation max (nm)	402	458	483	508	555	588
Emission max (nm)	457	480	506	524	584	633
Quantum yield	0.63	0.57	0.6	0.62	0.48	0.40
Extinction coefficient (M <sup>-1</sup> cm <sup>-1</sup> )	52 000	37 000	56 500	50 000	100 000	62 500
Brightness*	32.8	21.1	33.9	31	48.0	25.0
Brightness, % of EGFP	99	64	105	94	148	74
pKa	2.7	4.7	5.0	5.5	3.8	5.4
Structure	monomer	monomer	monomer	monomer	monomer	monomer
Cell toxicity	not observed	not observed	not observed	not observed	not observed	not observed
Aggregation	no	no	no	no	no	no
Maturation rate at 37°C	fast	fast	fast	fast	fast	fast
Molecular weight (kDa)	26	27	27	27	27	26

\*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

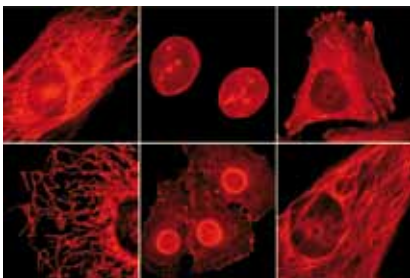
\*\*For stable cell line generation and organelle labeling we recommend that you use PhiYFP.

## FusionRed Fluorescent Protein

- Supermonomeric red fluorescent protein
- Superior performance in fusions
- Low cytotoxicity
- Fast maturation, high pH-stability and photostability

FusionRed is a red fluorescent protein characterized by improved performance in fusions and low toxicity. It lacks the residual tendency of other monomeric RFPs to dimerize at high concentration and behaves as a pure monomer at concentrations up to 10 mg/ml in HPLC analysis. Such „supermonomeric“ properties ensure superior efficiency of FusionRed in protein labeling applications, especially in cells with high expression level.

Similarly to parental mKate2, FusionRed demonstrates a fast maturation rate, high pH-stability and photostability, and significantly lower cytotoxicity than widely used mCherry and mRuby. FusionRed can be easily expressed and detected in a wide range of organisms and is mainly intended for protein labeling and long-term experiments including generation of transgenic animals.



Mammalian cells transiently transfected with FusionRed expression vectors produce bright fluorescence within 10-12 hours after transfection.

	FusionRed
Fluorescence color	red
Excitation max (nm)	580
Emission max (nm)	608
Quantum yield	0.19
Extinction coefficient ( $M^{-1}cm^{-1}$ )	94 500
Brightness*	18.0
Brightness, % of EGFP	53
pKa	4.6
Structure	supermonomer**
Cell toxicity	not observed
Aggregation	no
Maturation half-time (min)	130
Molecular weight (kDa)	26
Polypeptide length (AA)	232
Photostability, widefield***	150
Photostability, confocal***	176

\* Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

\*\* Purified recombinant protein behaves as a pure monomer at concentrations of 10 mg/ml as verified by high-performance liquid chromatography (HPLC)

\*\*\* Time to bleach 50% of fluorescent signal brightness.

[www.biocat.com/fusionred](http://www.biocat.com/fusionred)

## Fluorescent Biosensors

- Monitor  $Ca^{2+}$  or  $H_2O_2$  concentration
- Direct expression in cells
- No exogenous chemical compounds or cofactors required
- High selectivity and sensitivity

Genetically-encoded fluorescent sensors such as HyPer and Case12 are complex chimeric proteins consisting of a fluorescent protein and a sensing polypeptide fused in a specific manner. The sensors demonstrate clearly detectable changes in fluorescence in response to corresponding cell events.

SENSOR	DESCRIPTION	RESPONSE	ADVANTAGES
HyPer	Ratiometric sensor for highly specific detection of intracellular hydrogen peroxide	Rise in $H_2O_2$ concentration leads to decrease in sensor excitation peak at 420 nm and proportional increase in excitation peak at 500 nm	<ul style="list-style-type: none"> <li>• Ratiometric measurement</li> <li>• High specificity and sensitivity</li> <li>• Capability of targeting the sensor to the specific cell compartments</li> </ul>
Case12	High dynamic range sensor for detection of intracellular $Ca^{2+}$ changes	Rise in $Ca^{2+}$ concentration leads to significant increase of the reporter's brightness	<ul style="list-style-type: none"> <li>• High dynamic range</li> <li>• Relatively high pH stability</li> <li>• Capability of targeting the sensor to the specific cell compartments</li> </ul>

Fluorescent sensors for the early detection of caspase-3 activity are also offered.

[www.biocat.com/biosensors](http://www.biocat.com/biosensors)

## Advanced Fluorescent Tools

Photoactivatable fluorescent proteins (PAFPs) represent an effective tool for monitoring cellular events. These reporters are capable of pronounced changes in their spectral properties in response to irradiation with light of a specific wavelength and intensity. **PA-TagRFP** and **KFP-Red** convert from a non-fluorescent (dark) to a bright fluorescent state (photoactivation), whereas **PS-CFP2** changes fluorescence color (photoswitching or photoconversion).

Photosensitizers like **KillerRed** are chromophores that generate reactive oxygen species (ROS) upon light irradiation. They can be used for precise inactivation of selected proteins in chromophore-assisted light inactivation (CALI) technique and for light-induced cell killing, for example in photodynamic therapy. A photoinducible cell cycle inhibitor, **ArrestRed**, is also available.

[www.biocat.com/fluorescent-proteins](http://www.biocat.com/fluorescent-proteins)

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