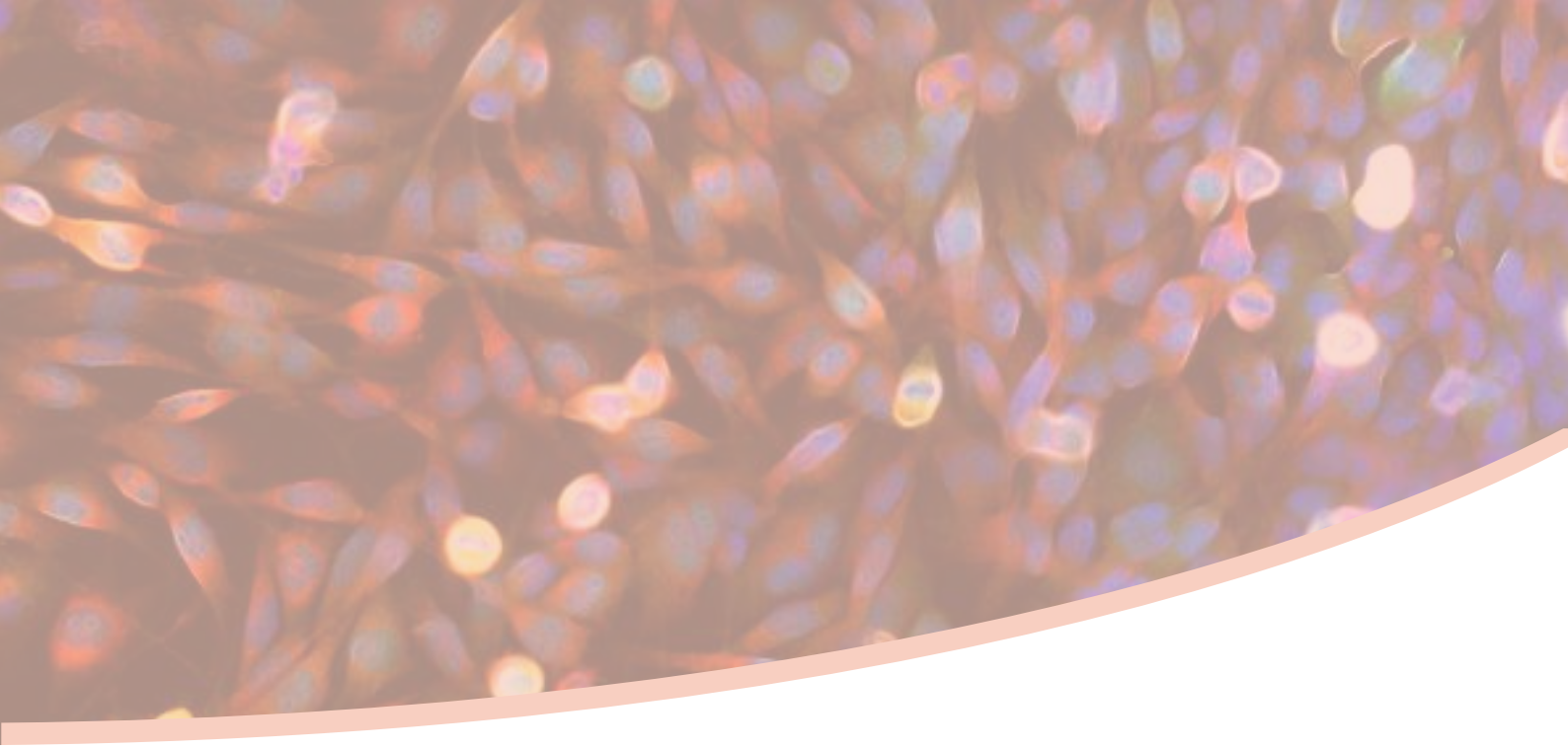


5. Cell Based Assay and Molecule Detection Kits

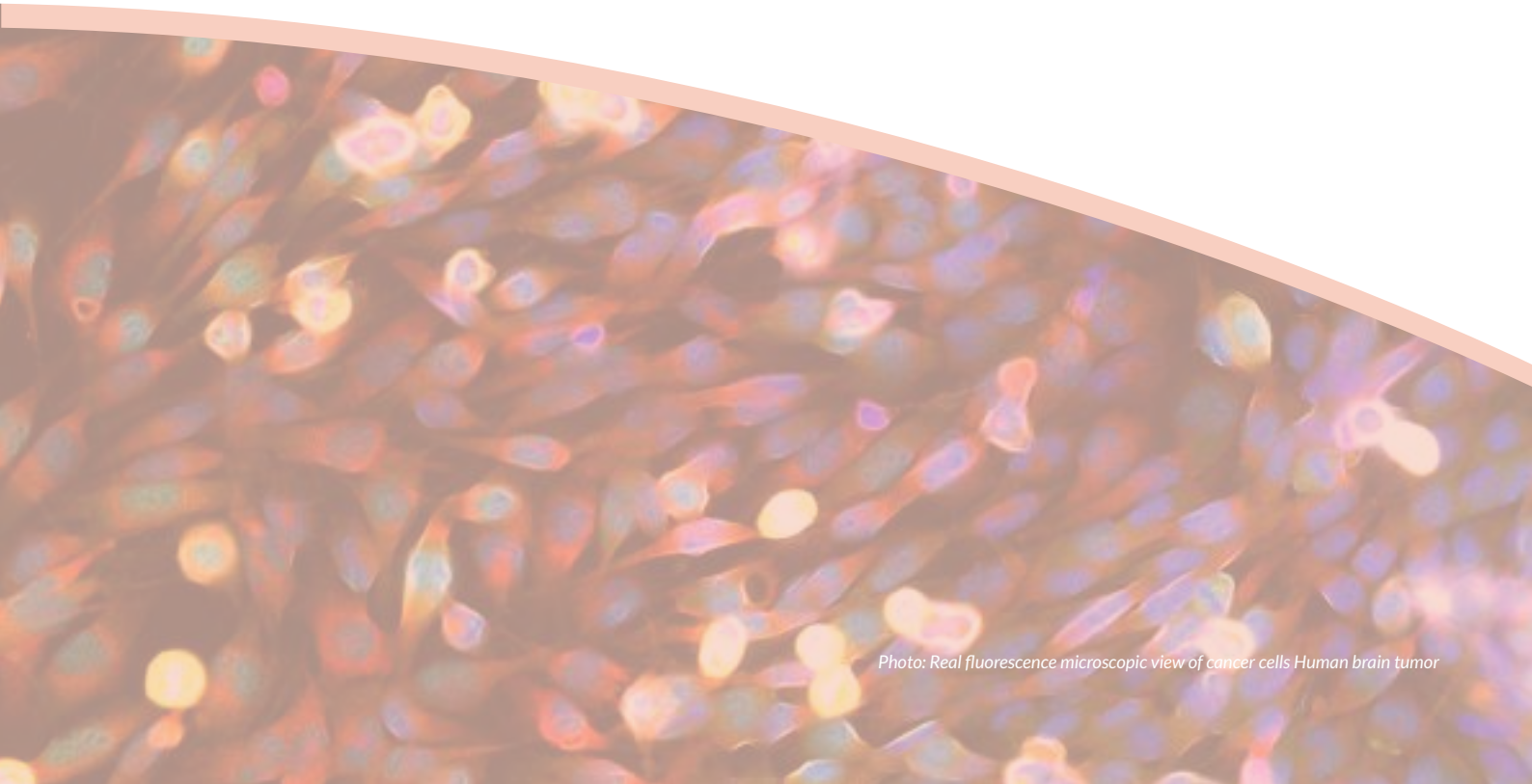


Photo: Real fluorescence microscopic view of cancer cells Human brain tumor

Cell Viability, Proliferation and Cytotoxicity assays

Annexin V Apoptosis Detection Kits

Ordering info:

Annexin V-FITC	
Cat No.	Size
CA011	100 assays
Annexin V-APC	
Cat No.	Size
CA012	100 assays
Annexin V-Biotin	
Cat No.	Size
CA013	100 assays
Annexin V-PE	
Cat No.	Size
CA014	100 assays

Description:

Annexin V Apoptosis Detection Kits is a convenient, easy-to-use and safe method for Apoptosis Detection. Annexins are a family of calcium-dependent phospholipid-binding proteins, which bind to phosphatidylserine (PS).

Externalization of phosphatidylserine residues on the outer plasma membrane of apoptotic cells allows detection via Annexin V. Once the apoptotic cells are bound with labelled Annexin V, it can be visualized with fluorescent microscopy or cytometry.

Since loss of membrane integrity is a pathognomonic feature of necrotic cell death, necrotic cells will stain with specific membrane-impermeant nucleic acid dyes such as propidium iodide, the membrane integrity of apoptotic cells can be demonstrated by the exclusion of these dyes.

Advantages & Features:

- ✓ **Easy and fast protocol.**
- ✓ **Versatile:** proven performance for both adherent and suspension cells.
- ✓ **Safe:** non-enzymatic assay that avoids fixation.

Includes for 100 assays:

- 500 µl Labeled Annexin V
- 50 mL Binding Buffer (10x)
- 500 µl Propidium iodide

Applications:

- ✓ Detect early/middle stages of apoptosis.
- ✓ Differentiate apoptosis from necrosis.

Related Products:

- XTT Cell Proliferation Assay Kit (p.78)

XTT Cell Proliferation Assay Kit

Ordering info:

Cat No.	Size
CA031	1,000 assays

Includes for 1,000 assays:

- 2 x 25 mL XTT Cell Proliferation Assay Kit Reagent
- 1 mL Activation Reagent

Related Products:

- SRB Cytotoxicidad assay (p.79)
- Resazurin Cell Viability assay (p.79)

Description:

XTT Cell Proliferation Assay Kit is an optimized, accurate and sensitive colorimetric assay that detects the cellular metabolic activities. During the assay, the yellow tetrazolium salt XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium) is reduced to a highly colored formazan dye by dehydrogenase enzymes in metabolically active cells.

This conversion only occurs in viable cells and thus, the amount of the formazan produced is proportional to viable cells in the sample. The formazan dye formed in the assay is soluble in aqueous solution and quantified by measuring the absorbance at wavelength 450 nm using a spectrophotometer. An electron coupling reagent, such as PMS (N-Methylphenazonium methyl sulphate), can significantly improve the efficiency of XTT reduction in cells.

Advantages & Features:

- ✓ **Accurate:** dye absorbance is proportional to the number of cells in each well.
- ✓ **Sensitive:** assayed even in low cell concentrations.
- ✓ **Fast protocol:** results within 2-5 hours with minimal handling steps.
- ✓ **Time-saving protocol:** avoids solubilisation step.
- ✓ **Complete solution:** includes all reagents needed for cell washing procedures.
- ✓ **Safe:** avoids radioactivity.
- ✓ **Optimized:** for high throughput assays (no requieres washing or other steps that can cause cell loss and variability).
- ✓ **Cost avoidance:** allows performance directly in a microtiter plate.

Applications:

- ✓ Spectrophotometric quantification of cell proliferation and viability in response to pharmaceutical, chemical, nutrients and environmental compounds.
- ✓ High throughput screening.

SRB Cytotoxicity Assay (Sulforhodamine B)

Ordering info:

Cat No.	Size
CA050	1,000 assays

Includes for 1,000 assays:

· 0.4 g SRB Dye
· 60 mL Fixative Reagent
· 100 mL Dye Wash Solution (10x)
· 200 mL SRB Solubization Buffer

Related Products:

· XTT Cell Proliferation Assay Kit (p.78)
· Resazurin Cell Viability assay (p.79)

Description:

Sulforhodamine B (SRB) Cytotoxicity Assay is a sensitive, reproducible and easy-to-use assay based on the ability of SRB to bind to protein components of cells that have been fixed to tissue culture plates. SRB is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic aminoacid residues under mild acidic conditions and dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass.

The fixed dye is solubilized and is measured photometrically at OD 540 nm with a reference filter of 690 nm. The OD values correlate with total protein content and therefore with cell number.

Advantages & Features:

- ✓ **Sensitive.**
- ✓ **Easy-to-use.**
- ✓ **Fast:** avoids time-sensitive measurement.
- ✓ **Reproducible.**
- ✓ **Great linearity.**
- ✓ **Good signal-to-noise ratio.**
- ✓ **Has a stable end-point.**

Applications:

- ✓ Detection of cell toxicity, death, viability or proliferation.
- ✓ High throughput screening.

Resazurin Cell Viability Assay

Ordering info:

Cat No.	Size
CA035	10,000 assays

Includes for 10,000 assays:

· 4 x 25 mL Resazurin solution

Related Products:

· XTT Cell Proliferation Assay Kit (p.78)
· SRB Cytotoxicidad assay (p.79)

Description:

Resazurin Cell Viability Assay is a reliable, sensitive and easy-to-use fluorescent assay that detects cellular metabolic activity. Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue dye non-fluorescent until it is irreversibly reduced to the pink colored and highly red fluorescent resorufin by dehydrogenase enzymes in metabolically active cells.

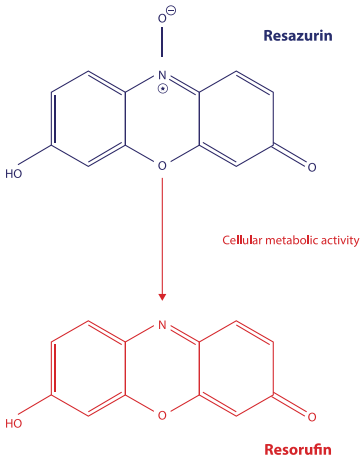
The fluorescent signal is monitored using 530-560 nm excitation wavelength and 590 nm emission wavelength. The absorbance is monitored at 570 nm and 600 nm. The fluorescent or colorimetric signal generated from the assay is proportional to the number of living cells in the sample.

Advantages & Features:

- ✓ **Easy procedure:** easy to perform with minimal handling.
- ✓ **Really fast:** just one step to results.
- ✓ **Reliable.**
- ✓ **Sensitive.**
- ✓ **Safe.**
- ✓ **Cost-effective.**

Applications:

- ✓ Spectrophotometric measurement of metabolic activity of living cells.



Senescence Detection Kit (SA-ß-gal Staining)

Ordering info:

Cat No.	Size
CA090	100 assays

Includes for 100 assays:

· 150 mg X-Gal (lyophilized)
· PBS (10x)
· Staining Solution A
· Staining Solution B
· Staining Solution C
· Fixative solution (10x)

Related Products:

· PBS (p.133)
· X-Gal (p.19)

Description:

Senescence detection kit is a fast, convenient and easy-to-use kit that measures activity of SA-B-Gal in cells cultures by hydrolysis of X-gal, which results in the accumulation of a distinctive blue color in senescent cells.

Senescence cells display a phenotype like increase of cell size, distinctive flat morphology, changes in gene expression and activity of senescence-associate ß-galactosidase (SA-ß-gal).

Senescence represent tumor suppressor mechanism for this reason cellular senescence has become an increasingly target in the development of novel therapeutics.

Advantages & Features:

- ✓ **Fast, convenient and easy procedure:** takes 28 minutes to results with minimal handling steps.
- ✓ **The specific histochemical marker is only present in senescent cells** and is not found in pre-senescent, quiescent or immortal cells.

Applications:

- ✓ Histochemically detect SA-β-Galactosidase activity in cultured cell and tissue sections.

Reporter Gene Assays

SEAP Reporter Gene Assay

Ordering info:

Cat No.	Size
CA040	288 assays

Includes for 288 assays:

· 3 x 96 W Solid Plate (white)
· 3 units of lid
· 50 µl Alkaline Phosphatase Standard
· 15 mL SEAP Substrate (Luminescence)

Related Products:

· FastCONTROL™ Dual Reporter Plasmid (p.28)
· CANFAST™ Transfection Reagent (p.76)

Description:

Secreted alkaline phosphatase (SEAP) reporter gene is an easy, sensitive and fast assay that utilizes enzyme activity of alkaline phosphatase to dephosphorylate the chemiluminescent Alkaline Phosphatase substrate into an unstable dioxetane anion which decomposes and emits light.

SEAP encodes a truncated form of the placental enzyme that lacks the membrane anchoring domain, thereby allowing the protein to secret efficiently from transfected cells.

Changes in levels of SEAP activity detected in the culture medium are directly proportional to changes in intracellular concentrations of SEAP mRNA and protein.

Advantages & Features:

- ✓ **Convenient:** single set of cells are used for both the SEAP assay and another purpose.
- ✓ **Time-saving protocol:** results in 55 minutes due the elimination of cell lysates preparation.
- ✓ **Cost-effective:** allows performance directly in a microtiter plate.
- ✓ **Sensitive:** assayed even in low cell concentrations.
- ✓ **Secreted** from transfected cells into the culture medium.

Applications:

- ✓ Measurement the levels of SEAP in the culture medium of transfected cells.

MUG - Galactosidase Assay kit

Ordering info:

Cat No.	Size
CA085	500 assays

Includes for 500 assays:

- 20 mM ß-galactosidase substrate 4MU
- 10 mM Reference Standard
- ß-galactosidase enzyme (0.1 mg/mL)
- Triton X-100
- 1M DTT
- Assay Buffer (2x)
- Stop solution

Description:

The MUG ß-Galactosidase Assay Kit is an efficient, easy and highly sensitive tool to measure levels of active ß-galactosidase expressed in cells transfected with plasmids expressing *Lac Z*.

Lac Z is often used as reporter gene in Transfection experiments because the ß-galactosidase is highly resistant to proteolytic degradation and its activity is easily measured. ß-galactosidase performs the hydrolysis of 4-methylumbelliferyl ß-D-galactopyranoside (MUG) to the 4-methylumbelliferone (4MU). This MUG produces as a bright blue fluorescence that are detected at excitation/emission = 360/460 nm. The concentration of ß-galactosidase is proportional to fluorescence produced.

Advantages & Features:

- **Fast, easy and convenient.**
- **Easy-to-use** method to quantify the enzyme expression in transfected cells.
- **Sensitive:** measure ß-galactosidase at femtogram level.

Applications:

- Measurement of β-Galactosidase activity in the lysates of transfected cell.

Related Products:

- PBS (p.133)
- CANFAST™ Transfection Reagent (p.76)
- ONPG – Galactosidase Assay kit (p.81)
- FastCONTROL™ Dual Reporter Plasmid (p.28)

ONPG - Galactosidase Assay kit

Ordering info:

Cat No.	Size
CA080	500 assays

Includes for 500 assays:

- ONPG Substrate solution
- DTT
- Buffer Lysis
- Buffer Assay
- Buffer Stop
- ß-galactosidase enzyme

Related Products:

- pOnebyOne™ Mammalian expression vectors (p.22)
- pColiExpress™ Glue Enzyme kits (p.34)
- FastCONTROL™ Dual Reporter Plasmid (p.28)
- Custom solutions (p.147)

Description:

The ONPG B-Galactosidase Assay Kit is an optimized, stable and colometric tool to fast measure the levels of active ß-galactosidase expressed in cells transfected with plasmids expressing *Lac Z*.

Lac Z is often used reporter gene in experiments transfection because the ß-galactosidase is very resistant to proteolytic degradation and its activity is easily measured. ß-galactosidase performs the hydrolysis of orthonitrophenyl-ß-D-galactopyranoside (ONPG) to the ortho-nitrophenol (ONP). This ONP produces as a bright yellow colour that is detected at absorbance 420 nm. The concentration of ß-galactosidase is proportional to colour produced.

Advantages & Features:

- **Proven performance** to quantify high expression level of beta-Gal.
- **Very stable:** resistant to proteolytic degradation and easily assayed.
- **Convenient** for all transfection assays.
- **Versatile:** proven performance for cultured cells and tissues.
- **Rapid and easy protocol.**
- **Cost-effective.**

Applications:

- Measurement of β-Galactosidase activity in the lysates of transfected cells.

Firefly Luciferase Assay Kit

Ordering info:

Cat No.	Size
CA130	100 assays
CA135	1,000 assays

Includes for 100 assays:

- 10 mL Luciferase Assay Substrate
- 4 mL Cell Lysis Buffer (5x)
- 10 µg Luciferase (control)

Related Products:

- pOnebyOne™ Mammalian expression vectors (p.22)
- Custom solutions (p.147)
- FastCONTROL™ Dual Reporter Plasmid (p.28)

Description:

Luciferase from the firefly (*Photinus pyralis*) is an accurate, sensitive and easy **Luciferase Assay Kit** for studying gene reporter regulation and function in transformed cell lines in culture.

Firefly luciferase has an apparent molecular weight of 62 kDa, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes the oxidation of reduced luciferin in the presence of ATP-Mg^{2+} and oxygen to generate CO_2, AMP, PPi, oxyluciferin and produces a flash of light that is proportional to the quantity of luciferase in the reaction mixture.

The Luciferase Assay Substrate includes coenzyme A, ATP and luciferin. Including coenzyme A in the reaction enhances the sensitivity of the assay and provides a sustained light reaction (half-life >5 minutes). This eliminates the need for automated luminometer injection of substrate and allows analysis by photographic film or scintillation counting.

Advantages & Features:

- **Easy and Fast protocol:** results within 14 minutes.
- **Sensitive and linear:** correlation between luciferase gene expression and light output for transfection.
- **Accurate.**
- **Ideal for high throughput assays.**

Applications:

- Detection and quantification of Firefly luciferase.

Stress oxidative Assay Kits

SOD Assay Kit

Ordering info:

Cat No.	Size
CA061	100 assays

Includes for 100 assays:

- 1 mL WST-1 Reagent
- 20 mL SOD Assay Buffer
- 10 mL SOD Dilution Buffer
- 20 µl SOD Enzyme solution
- 50 µl SOD Standard (50 U/µl)

Related Products:

- PBS (p.133)
- Custom solutions (p.147)

Description:

Superoxide dismutases (SOD) catalyse the breakdown of superoxide radicals and provide the first line of defense against oxygen toxicity.

SOD Assay Kit, superoxide ions are generated from the conversion of xanthine and O_2 to uric acid and H_2O_2 by Xanthine Oxidase (XO). The superoxide anion then coverts the tetrazolium salt WST-1 to the colored product WST-1 formazan.

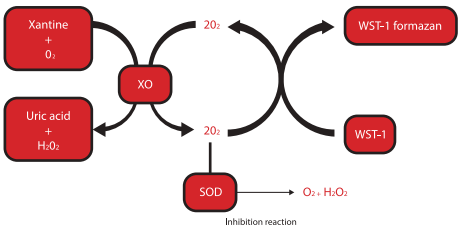
Absorbance is then measured at 450 nm using a standard microplate reader. Addition of SOD to this reaction reduces superoxide ion levels, thereby lowering the rate of WST-1 formazan formation. SOD activity in the experimental sample is measured as the percent inhibition of the rate of WST-1 formazan formation.

Advantages & Features:

- **Easy-to-use.**
- **Fast protocol.**

Applications:

- Quantitative determination of superoxide dismutase (SOD) enzyme activity.



Catalase activity Assay Kit

Description:

Catalase Activity Assay Kit is a fast, easy and highly sensitive method for measuring catalase activity in biological samples.

In the assay, catalase first reacts with H_2O_2 to produce water and oxygen. In the presence of horseradish peroxidase (HRP), the unconverted H_2O_2 reacts 1:1 with the fluorogenic substrate 10-Acetyl-3,7- dihydroxyphenoxazine to produce a product highly fluorescent (resorufin), which is measured at Ex/Em=535/587nm (fluorometric method) or at 570 nm (colorimetric method).

Applications:

- ✓ Determination of catalase activity by colometric or fluorometric assay.

Advantages & Features:

- ✓ **Fast and easy protocol:** it takes 34 minutes.
- ✓ **Sensitive** assays for measuring catalase in various biological samples.

Ordering info:

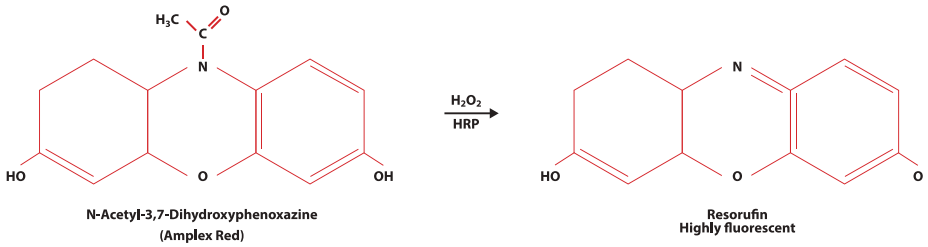
Cat No.	Size
CA063	100 assays

Includes for 100 assays:

- 200 µl Probe (in DMSO)
- 20 mL CAT Assay Buffer
- 50 µl H_2O_2 (0.88 M)
- 250 µl HRP solution
- 1.5 mL Stop solution
- 5 µl Catalase Positive Control

Related Products:

- PBS (p.133)
- Custom solutions (p.147)



N-Acetyl-3,7-Dihydroxyphenoxazine (Amplex Red)

Resorufin Highly fluorescent

Glutathione Assay Kit

Description:

Glutathione Assay Kit is an accurate, fast and easy-to-use assay based on a chemical reaction in two steps. The Kit makes possible the quantification of glutathione with only one sampling and one colorimetric measurement.

Glutathione (gamma-glutamyl-cysteinyl-glycine, GSH) is a cysteine-containing tripeptide, which is the most abundant nonprotein thiol in cells. GSH is composed of glutamate, cysteine, and glycine and is synthesized in both eukaryotic as well as prokaryotic cells. It is a powerful antioxidant that prevents ROS-mediated damage to essential cellular components and acts as a cofactor for enzymes in the destruction of ROS.

Applications:

- ✓ For quantitative determination of reduced Glutathione (GSH).

Advantages & Features:

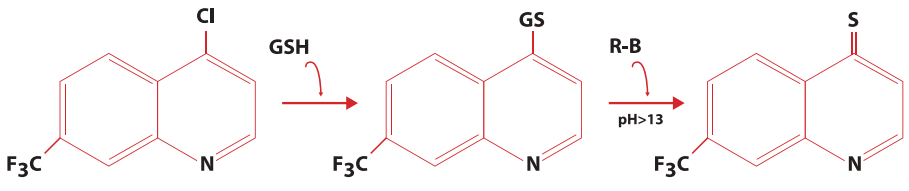
- ✓ **Cost avoidance:** avoids the use of any enzyme as reagent.
- ✓ **Really fast and easy procedure.**
- ✓ **Accurate:** more specific than the DTNB method.

Ordering info:

Cat No.	Size
CA066	100 assays

Includes for 100 assays:

- 6 mL Reagent A (R-A)
- 10 mL Reagent B (R-B)
- 2 x 50 mL Buffer Solution
- 2 mg GSH Standard
- 3 x 0.5 mg Metaphosphoric Acid



R-A Thioether