Intracellular Ca^{++} levels have become important indicators for the activation state of ion channels and G-protein coupled receptors as well as for the phases of apoptosis and cell injury. Though the respective kinetics and the absolute amounts of the Calcium levels are different for each of these physiological processes there are common ways for monitoring them. Luminescent labels like Aequorin as well as fluorescent ones are versatile and widely used solutions for microplate assays. Fura 2 and Indo-1 provide ratiometric readout thereby reducing effects caused by leaking or bleached dyes or varying assay conditions.

Product name	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em}$ max. [(a)] Free Ca^{2+}(nm)	$\lambda_{exc}/\lambda_{em}$ max. [(a)] High Ca^{2+} (nm)	Kd Ca^{2+} (nM)	Applications
Fluo-3 AM[(b)]	1129	503 / weak	505 / 526	390	. Most classical calcium indicator
Fluo-8 AM	1000	490 / weak	490 / 514	389	. No Wash step needed . 4 times brighter than Fluo-3 . Loading at room temperature
Fluo-8H AM	1100	490 / weak	490 / 514	232	. High Ca^{2+} concentration indicator
Fluo-8L AM	1100	490 / weak	490 / 514	1 860	. Low Ca^{2+} concentration indicator
Rhod-4 AM		530 / weak	530 / 555	525	. Red calcium indicator . 4 times brighter and 10 times larger windows assay than Rhod-2 . Loading at room temperature
Fura-PE3 AM	1258	335 / 495	380 / 495	250	. Leakage resistant form of Fura-2 . Ratio of reads with 2 different $\lambda_{ex.}$. Avoids interference due to dye distribution and photobleaching
Indo-PE3 AM	1266	338 / 480	338 / 410	260	. Leakage resistant form of Indo-1 . Ratio of reads with 2 different $\lambda_{em.}$. Avoids interference due to dye distribution and photobleaching

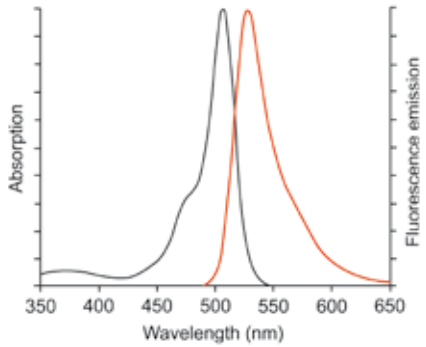
(a) after hydrolysis
(b) **AM ester** are membrane-permeant and thus increases greatly cell loading that can be performed by simple incubation of the cells or tissue preparation in a buffer containing the AM ester. Pluronic® F-127, a mild non-ionic detergent, can facilitate AM esters loading. The AM esters themselves do not bind to Ca^{2+}. However, once they have entered the cells, they are rapidly hydrolyzed by intracellular esterases into the parent Ca^{2+} compounds, thus becoming fully fluorescent upon binding to Ca^{2+}.
Many other ion indicators are available in our Interchim' range of dye. Please contact us for specific application needs.

Fluo-3 AM

Standard Ca^{2+} concentration indicator

- Large dynamic range
- Low compartmentalization
- Appropriate apparent Ca^{2+} binding affinity

Description	P/N :	Qty
Fluo-3 AM	**FP-78932A**	1 mg
$\lambda_{exc.}/\lambda_{em.}$ = 505/523 nm	**FP-R1245A**	1 mg FluoProbes Pure Grade
Kd = 390 nm	**FP-78932B**	10 x 100 µg
	FP-78932C	20 x 50 µg
	FP-M2036A	1 ml (1mM solution in DMSO)
	FP-78932D	50 mg

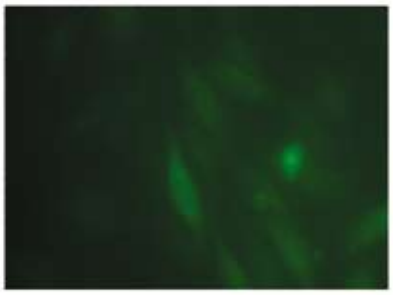


Fluo-3 has an absorption spectrum compatible with excitation at 488 nm by argon-ion laser sources, and a >100 fold fluorescence intensity increase in response to Ca^{2+} binding. Fluo-3 proves to be the generally most applicable Ca^{2+} indicator, even if it is more susceptible to photobleaching than many of the other Ca^{2+} indicators.

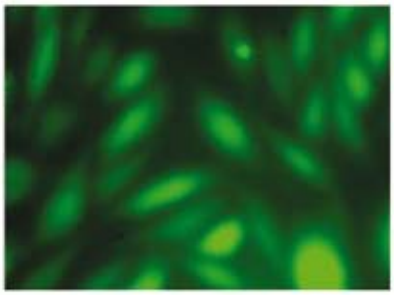
Fluo-8 AM - NoWash

The next generation calcium indicator for automated screening (HTS) applications

Fluo-3



Fluo-4

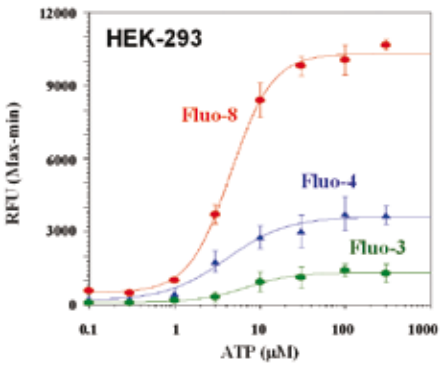


Fluo-8

- **Increased signal intensity**
- **Rapid dye loading** : dye loading at RT (rather than 37°C required for Fluo-4 AM)
- **Convenient and robust** : No wash step needed.
- Performed in 96 or 384-well microtiter-plate

The **Fluo-8 NW** (No Wash) can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Fluo-8 NW are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells and its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which greatly increase the fluorescence of Fluo-8 NW. The characteristics of its long wavelength (490/514 nm), high sensitivity, and >100 times fluorescence enhancement (when it forms a complex with calcium) make Fluo-8 NW an ideal indicator for measurement of cellular calcium.

Description	P/N :	Qty
Fluo-8 No Wash Calcium Assay Kit, Medium removal[1]	**CJ2560**	10 plates
	CJ2561	100 plates
Fluo-8 No Wash Calcium Assay Kit, 1% FBS Medium[2]	**CJ2550**	10 plates
	CJ2551	100 plates
Fluo-8 AM	**CP7501**	5 x 50 µg
$\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}) : 505/523 nm ; Kd(Ca^{2+}) = 390 nM ; MW : 1000		
Related products :		
Probenecid, Cell culture tested, to suppress efflux of dyes	**FP-288652**	10 x 150 mg
Probenecid, water soluble	**FP-288653**	10 x 150 mg
Ionomycin, Ca^{2+} ionophore	**FP-53989A**	1 mg
Ionomycin, Ca^{2+} ionophore	**FP-53989B**	5 mg



ATP dose responses in HEK-293 cells

Note 1 : It is important to remove the growth medium in order to minimize background fluorescence, and compound interference with serum or culture media.
Note 2 : Alternatively, one can grow the cells in growth medium with 0.5-to 1% FBS to avoid medium removal step.

■ Fluo-8H AM

High cytosolic Ca^{2+} concentration indicator

High Ca^{2+} concentrations, present in some organelles (mitochondria, vacuoles) and in excitable cells (fibroblast i.e.), were hardly detected : standard dyes Fluo-3, Fluo-4 and Rhod-2 have too high affinity for Ca^{2+}.

The measurement of cytosolic free Ca^{2+} ion concentration with low affinity Ca^{2+} indicators has advantages for kinetic studies of cytosolic [Ca^{2+}] transients when compared with more commonly used high affinity Ca^{2+} indicators. Their dynamic range and linearity are better suited to measurement of high-localised transient concentration changes that exist near sites of influx or release, and the additional buffering introduced by the indicator is minimised.

Description	P/N :	Qty
Fluo-8H AM (490/514 nm)	**FP-CP7531**	10 x 50 µg
$\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}): 490/514 nm ; Kd(Ca^{2+})=232 nM ; MW : 1100	**FP-CP7530**	1 mg
Related products		
Mag-Fura-2 AM ($\lambda_{exc.}/\lambda_{em.}$: 369, 329/510 nm)	**FP-35374C**	20 x 50 µg

■ Fluo-8L AM

Low cytosolic Ca^{2+} concentration indicator

Description	P/N :	Qty
Fluo-8L AM	**FP-CP7551**	10 x 50 µg
$\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}): 490/514 nm ; Kd(Ca^{2+})=1.86 µM	**FP-CP7550**	1 mg

■ Rhod-4™ AM - No Wash (NW)

The brighest red fluorescent calcium indicator

Rhod-2 is most commonly used among the red fluorescent calcium indicators. However, Rhod-2 AM is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. **Rhod-4™** has been developed to **improve cell loading** and calcium response while maintaining the spectral wavelength of Rhod-2. In CHO and HEK cells Rhod-4™ AM has cellular calcium response that is 10 times more sensitive than Rhod-2 AM.

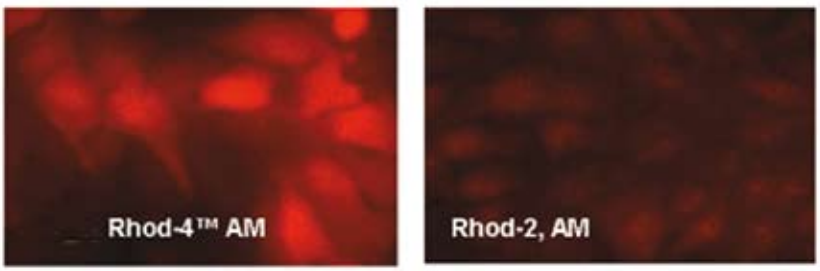


Figure 2. Rhod-4 AM vs Rhod-2 AM in U2OS. U2OS cells were seeded overnight at 40 000 cells per 100 µl per well in a 96 wells black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 µl of 5uM Rhod-4 AM or Rhod-2 AM in HHBS at 37°C, 5% CO^2 incubator for 1 hour. The cells were washed with 2 times with 200 µl HHBS, then imaged under fluorescent microscope using Tritc channel.

Rhod-4™ is the brightest red calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Rhod-4™ are cleaved by non-specific cell esterase, resulting in a negatively charged fluorescent dye that stays inside cells, and its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with screening compounds, the receptor signals release of intracellular calcium, which greatly increase the fluorescence of Rhod-4. The characteristics of its long wavelength, high sensitivity, and >250 times fluorescence increases (when it forms complexes with calcium) make Rhod-4™ an ideal indicator for measurement of cellular calcium. This Rhod-4 NW Calcium Assay Kit provides an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

The **Rhod-4 NW Calcium Assay Kit** provides a homogeneous fluorescence-based assay for detecting the intracellular calcium mobilization, and a preferred method in drug discovery for screening. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with our proprietary Rhod-4 NW which can cross cell membrane.

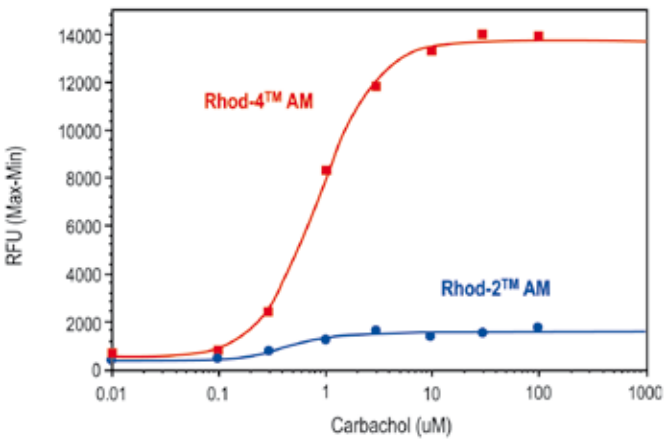


Figure 1. Carbachol Dose Response in HEK-293 cells measured with Rhod-4 NW Calcium Assay kit and Rhod-2 AM. HEK-293 cells were seeded overnight at 40 000 cells per 100 µl per well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 µl of the Rhod-4 NW calcium assay kit, or 5 µM Rhod-2 AM at 37°C, 5% CO_2 incubator for 1 hour. Carbachol (25 µl/well) was added by NOVOstar (BMG LabTech) to achieve the final indicated concentrations. The EC^{59} of rhod-4 NW is about 0.8 uM.

Description	P/N :	Qty
Rhod-4™ NW Calcium Assay Kit, Medium Removal	**CQ6080**	1 plate
	CQ6081	10 plates
	CQ6082	100 plates
Rhod-4™ NW Calcium Assay Kit, 1% FBS Growth Medium	**CQ6090**	1 plate
	CQ6091	10 plates
	CQ6092	100 plates
Rhod-4™ AM $\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}) : 530/555 nm ; Kd(Ca^{2+}) = 525 nm	**CQ6061**	5 x 50 µg
	CQ6062	10 x 50 µg
	CQ6063	20 x 50 µg
	CQ6064	1 mg

■ Fura-PE3 AM & Fura-2 AM

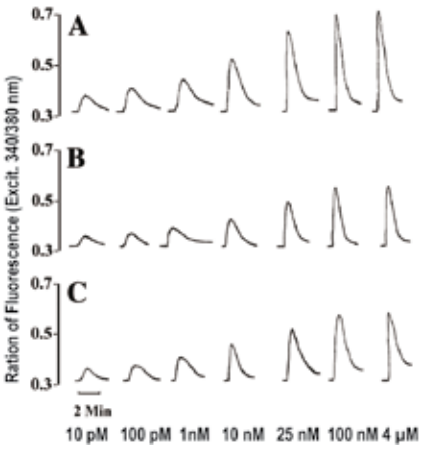
Leakage resistant form of Fura-2, a popular Setrometric Ca^{2+} indicator

Fura-2 AM may be useful in microplate studies, where cell lines with different properties are compared or where screening treatments lead to differences in the number of cells or dye loading. Some of the limitations in the use of Fura-2 appear to be overcome by the use of glass bottom microplates (See page 78).

Reference : Robinson JA et al., Ratiometric and non-ratiometric Ca^{2+} indicators for the assessment of intracellular free Ca^{2+} in a breast cancer cell line using a fluorescence microplate reader, J Biochem Biophys Methods. 2004 Mar 31;58(3):227-37.

Fura-PE3 is an improved version of Fura-2, that reduces cell leakage and thus increases dye loading accuracy.

Description	P/N :	Qty
Fura-2 AM	**FP-42776A**	1 mg
	FP-42776C	20 x 50 µg
	FP-85312A	1 ml (1 mM)
Fura-PE3 AM	**FP-AM603A**	500 µg



Representative experiment of angiotensin II (ANG II)-evoked changes in the ratio of fura 2 fluoresence (340/380 nm) in adherent neonatal rat cardiomyocytes (NRC). Primary cultures of NRC loaded with Fura 2 were stimulated with increasing concentrations of ANG II (10 pM-1 µM) either in the absence (control ; A) or presence of AA-861 (10 µM ; B) or MK-571 (100 nM ; C).

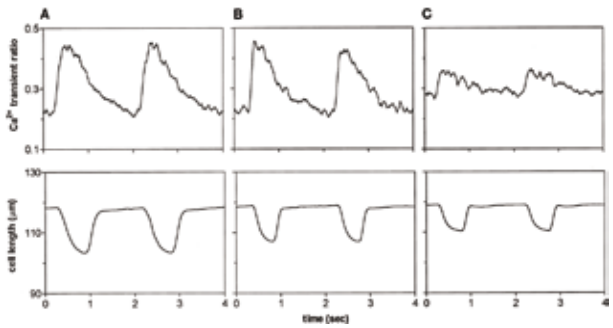
■ Indo-1 AM

Indo-1 AM has a shift in the emission from 485 nm to 405 nm in the presence of calcium. Indo-1 AM can be used with a single argon-ion laser for excitation and to monitor two different emissions.

Description	P/N :	Qty
Indo-1 AM	**FP-427755**	500 mg
	FP-42775A	20 x 50 µg
	FP-98180A	1 ml (1 mM)
Related products :		
4-bromo A-23187, Ca^{2+} ionophore for UV light-excited dyes	**FP-372221**	1 mg
A-23187 (calcimycin or Calcium Ionophore III) to equilibrate intracellular and extracellular $[Ca^{2+}]$	**FP-28362B**	5 mg

Related product

Description	P/N :	Qty
Calcium Calibration kit	**FP-21527A**	1 kit
Pluronic® F-127	**FP-37361A**	1 g
Pluronic® F-127, 20% solution (in DMSO)	**FP-69806A**	1 ml



Representative tracings from indo 1-loaded myocytes show simultaneous Ca^{2+} transients (top panels) and cell length (bottom panels). Myocytes were stimulated at 0.5 Hz after 6 h of no treatment (control) (A), LPS (10 ng/ml) (B), or LPS with ANG II (100 nM) (C).

■ Indo-PE3 AM

Leakage resistant form of Indo-1

Description	P/N :	Qty
Indo-PE3 AM	**FP-AM602A**	500 µg

Cell counting, viability & proliferation

Cell counting is required

- To monitor cells during cell cultures
- For cell preparation or any cell experiment
- To standardize cell samples for analysis.
- Cell proliferation
- Cytotoxicity assays

Several methods have been proposed, each fitting more or less to each specific application : counting dead cells may be acceptable for the preparation of cell extracts or desired when one do not want to operate with hazardous cells or for cytotoxicity study. At the opposite dead cells counting is generally precluded for cell culture and bioassays. It may be useful to quantitate only viable cells, or only fast proliferating cells.

Interchim provides a large choice of cell assays covering standard as well as innovative methods for general to specific cell assays.

Technical tip

MicroPlate readers & Imaging systems

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with instruments.

BERTHOLD TECHNOLOGIES

***NightOWL LB983 NC100**

***Mithras LB940 MultiMode Reader**

Selection guide

Probe	Principle	Detection Method	Dead	Viable	Proliferating	Features/Advantages - Drawbacks
Trypan blue	Membrane exclusion	Colorimetric Microscopy	++	++	++	Cheap, but time consuming, not scalable. Do not state on viability.
Hoechst	DNA probe exclusion	Fluorimetric	++	++	+++	Cheap, Scalable, Non toxic. Do not state on viability. More rapid than MTT/XTT ; unfixed or fixed samples.
MTT	Formazan dye, orange precipitate.	Colorimetric	-	++	+++	Popular method. Sensitive, Scalable. Non toxic Increased solubility and performance from MTT to XTT and WST.
XTT	Same as MTT but more soluble.	Colorimetric	-	++	+++	
WST	Formazan dye, soluble & not toxic		-	++	+++	
UptiBlue	ratiometric blue probe for cell redox	Colorimetric Fluorimetric	-	+++	+++	No solubilization step (unlike MTT). Applyalso to adherent cells. Sensitivity similar to MTT/XTT, but easier to use Fluorimetry/Superior sensitivity to MTT / XTT.
Calcein-AM	Calcein accumulation in cytoplasm	Fluorimetric	-	+++	++	No solubilization step (unlike MTT/XTT). Adaptable to a wide variety of techniques, including : microplate assays, in vivo cell tracing. Do not work for bacteria. May alter some cell functions.
GAPDH	Release of GAPDH coupled to ATP assay	Bioluminescence	-	+++	+	Measurement of Cell-Mediated (T Cells, ADCC, NK) or Complement-Mediated Cytolsis.
CFSE	Fluorescein protein labeling	Fluorimetric	++	++	++	Useful when other method do not work properly. Do not state on viability.
AnnexinV	AnnexinV/PhosphoSerine	Fluorimetric	+	+++	+	Useful for Apoptosis study.
LDH	convertion in colored product		-	++	+	Recommended for cytotoxicity assays Serum Interference.
Luciferin Syst.	ATP measure	Luminescence	-	+	+++	Pros : sensitivity / linearity. Cons : signal depends on each cell line, on temperature
-3H Thymidine	DNA incorporation of radioactivity	Radioactivity	-	+	+++	Cons : hazardous (radioelements).
BRDU	DNA incorporation	Immunoassay	-	+	+++	
^{51}Cr release EU^{3+}	Release of radioactivity by cytoplasm	Radioactivity	-	-	+++	Recommended for cytotoxicity assays. Cons : hazardous (radioelements).
Propidium Iodide, AAD	**7-**Membrane permeability	Fluorimetric	+++	-	-	Used in combinaison of green fluorescence dye like. Annexin V-FP488 to discriminate dead cells from alive cells.