FLUOFORTE®
Calcium assay kit

A Brighter, More Robust Fluorescent Assay for Calcium Mobilization

F LUOFORTE® Calcium Assay Kits detect mobilization of intracellular calcium utilizing a fluorogenic calcium-binding dye optimized for superior cell-permeability and retention. The self-quenching dye undergoes an electronic change upon binding of calcium, resulting in a several order of magnitude greater fluorescence.

Mechanism of Action
The fluorogenic calcium-binding dye is provided to the cells as an acetoxymethyl (AM) ester, which is cell-permeable. Once inside the cells, the dye is hydrolyzed by intracellular esterases, which leads to generation of a cell membrane impermeable negatively charged form. In the absence of calcium, the calcium-binding moiety portion of the probe quenches the fluorescence of the fluorophore portion of the probe by photo-induced electron transfer. Binding of calcium relieves quenching and results in a several order of magnitude increase in the fluorescence emission intensity, with no shift in wavelength. The dye is capable of binding to physiologically relevant levels of calcium and increases in intracellular calcium lead to an increased fluorescence signal, which is readily measurable.

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Ordering Information

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ENZ-51017	10x96 tests

Manuals, SDS & CofA

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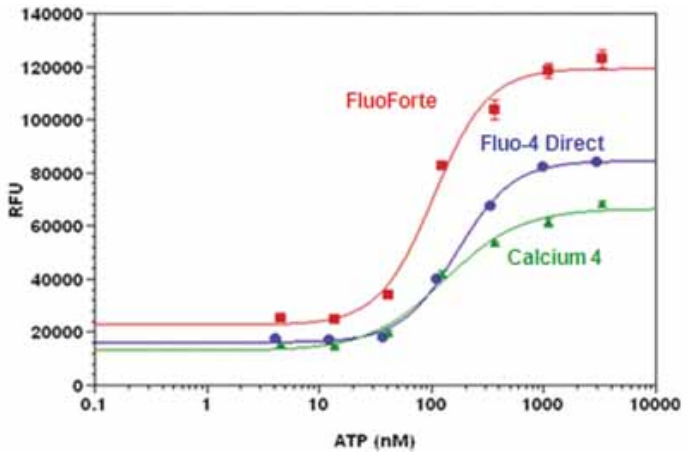
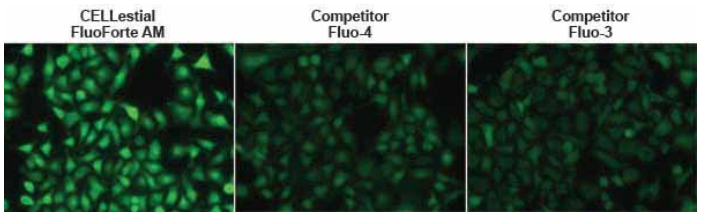
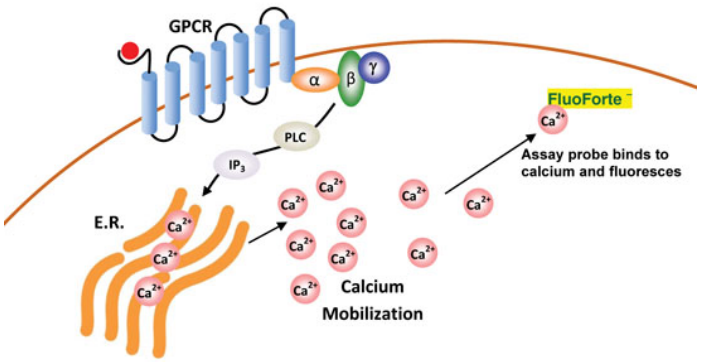
- Dye optimized for superior cell-permeability and retention
- Economical alternative developed for use with conventional dual-dispensing microplate readers, i.e. BioTek, BMG Labtech, etc.
- Provides EC50 values comparable to Fluo-4 and Calcium 4
- 2-fold brighter fluorescence vs. Fluo-4
- Sensitive dye provides larger assay window allowing for detection of even weak signal compound responses
- Better able to detect native levels of GPCR expression in cultured cells
- Data suitable for comparison with that obtained using alternative dyes



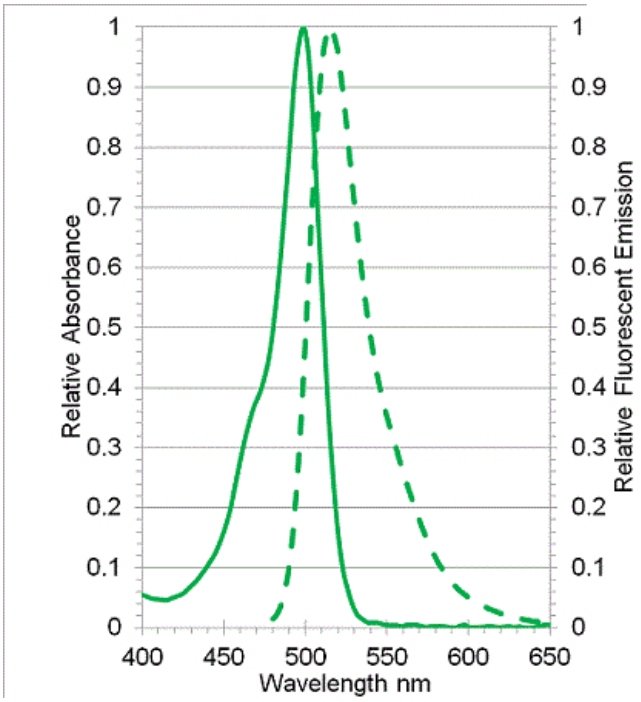
Obtain robust signal intensity. ATP dose response curves in CHO-M1 cells, expressing P2Y endogenous receptors. CHO cells were seeded overnight at 40,000 cells per 100 µl per well in a 96-well black wall/clear bottom microplate. The cells were incubated with 100 µl of Life Technologies' Fluo-4 Direct™ kit, Molecular Devices' Calcium 4 kit (both based upon manufacturer's protocol) or FLUOFORTE® dye. ATP (20µl/well) was added by FlexStation to achieve the final indicated concentrations. Comparable ATP EC50 values were obtained using all three dyes, while FLUOFORTE® generated the highest intensity signal.



FLUOFORTE® exhibits significantly brighter fluorescence intensity than Fluo-4 AM and Fluo-3 AM. Evaluation of relative dye fluorescence. U2OS cells were seeded overnight, growth medium was removed, and cells were incubated with 100 µl of FLUOFORTE® AM, Fluo-3 AM, or Fluo-4 AM in HHBS at 37 °C, 5% CO2 incubator for 1 hour. The cells were washed twice with 200 µl HHBS, and ATP (20 µL/well) was added to achieve concentrations of 200 nM with dye efflux inhibitor. Cells were then immediately imaged with a fluorescence microscope (Olympus IX71) using FITC channel.



FLUOFORTE® dye increases in fluorescent intensity when bound to calcium.



Excitation (solid line) and emission (dotted line) spectra of FLUOFORTE® dye

Handling & Storage

Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-20°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	Flow Cytometry, Fluorescence microscopy, Fluorescent detection, HTS
Application Notes	Provides a homogeneous fluorescence-based assay for detecting intracellular calcium mobilization across a broad spectrum of biological targets
Contents	**Reagent A** (lyophilized FLUOFORTE® dye), 1 vial **Reagent B** (dye efflux inhibitor), 10 x 1 ml **Reagent C** (Hanks' buffer with 20mm HEPES), 100 ml
Quality Control	% purity of Reagent A (FLUOFORTE® dye) by HPLC is ≥95%.
Quantity	For 10 x 96-well plates
Technical Info / Product Notes	Measurement of transient calcium mobilization from intracellular stores in response to the activation of G protein-coupled receptors (GPCRs) is considered a standard approach to the pharmacological characterization of receptors and compounds, frequently implemented in primary screening and lead development programs. The FLUOFORTE® Calcium Assay Kit is a member of the CELLESTIAL® product line, reagents and assay kits comprising fluorescent molecular probes that have been extensively benchmarked for live cell analysis applications.

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