

FluoForte™ CALCIUM ASSAY KIT

BRILLIANT RESULTS FOR CALCIUM ASSAY DISCOVERY



New Screening and Optimization Strategies in GPCR-Based Drug Discovery

Drugs targeting members of the G protein-coupled receptor (GPCR) super-family represent the core of modern medicine [1]. With estimated 600-1,000 members, the GPCR super family constitutes one of the largest families of molecular targets applicable to drug discovery in the human genome. They account for the majority of the best-selling drugs and roughly 40% of all prescription pharmaceuticals on the market today. Consequently, GPCRs are the target of choice for numerous high-throughput screening (HTS) campaigns designed to search for new drug leads of potential therapeutic significance [2].

Among fluorescence-based assays, measurement of transient calcium mobilization from intracellular stores in response to the activation of $G\alpha_q$ coupled GPCRs is considered a standard approach to the pharmacological characterization of receptors and compounds and is frequently implemented in primary screening, as well as to generate a compound structure activity relation (SAR) for lead development programs. Ease of use and relatively low cost have been the primary motivations for the increased use of intracellular calcium assays in GPCR drug discovery. The basic principle of the cell-based assay is illustrated in Figure 1.

LIT: [1] Drug design strategies for targeting G-protein-coupled receptors: T. Klabunde & G. Hessler; *ChemBioChem* **3**, 928 (2002) ▪ [2] FLIPR assays of intracellular calcium in GPCR drug discovery: K. B. Hansen & H. Brauner-Osborne; *Methods Mol. Biol.* **552**, 269 (2009)

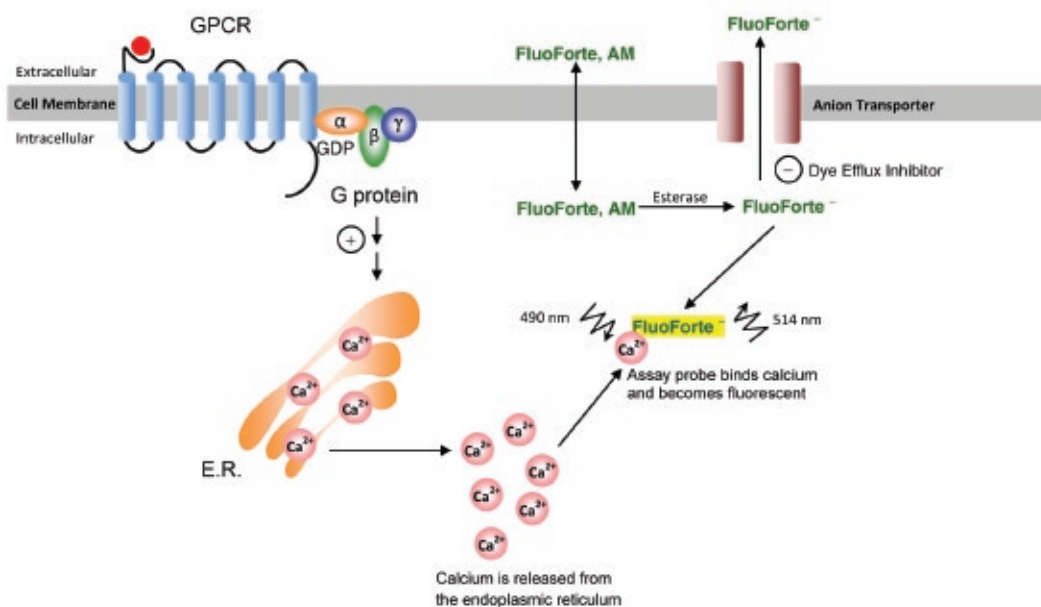


FIGURE 1: Principle of the intracellular calcium mobilization assay in living mammalian cells, based upon the fluorescent calcium indicator dye, FluoForte™ reagent. FluoForte™ reagent enters the cell as a membrane permeable acetoxyethyl (AM) ester. Once inside the cell, the dye is hydrolyzed (cleaved) by intracellular esterases. The resulting cell membrane-impermeable, negatively charged form of the dye is now capable of binding to calcium ions that are released from intracellular stores upon activation of the GPCR. Some cells express an organic anion transporter, which actively exports the dye outside of the cell, leading to decreased fluorescence signal. This is counteracted by adding Dye Efflux Inhibitor. Besides its robust applications for GPCR targets, our FluoForte™ Calcium Assay Kit can also be used for characterizing calcium ion channels and screening calcium ion channel-targeted compounds.

FluoForte™ Calcium Assay Kits

FluoForte™ Calcium Assay Kits are the brightest and most sensitive fluorescent Ca^{2+} indicators to detect calcium mobilization across a broad spectrum of biological targets. With much higher signal intensity, FluoForte™ dye offers superior measurement of challenging cell lines and receptors. EC_{50} values (comparable to Fluo-3 and Fluo-4 dyes) and assay reproducibility have been benchmarked upon activation of a variety of GPCRs. FluoForte™ is more than 2-fold brighter than Fluo-4, yields more robust signal (Z' -factor ≥ 0.75) and provides a larger assay window; making it suitable even when compounds elicit only a weak signal response. Based upon the new fluorescent probe, FluoForte™ Calcium Assay Kit is highly efficient, amenable to automation and provides a robust approach to screening GPCR agonists that mobilize calcium upon activation.

- Homogeneous mix-and-read, no-wash calcium mobilization assay
- Load dye in live cells at 37°C or even room temperature for easier automation
- Broadly applicable to both GPCR and calcium ion channel targets
- Significantly brighter fluorescence intensity than competitors' no-wash kits
- Provides comparable EC_{50} values as obtained using Fluo-3, Fluo-4 and FLIPR® Calcium 4 assay kits
- No quencher dye, so no potential interference from compound ligand-receptor interactions
- Larger assay window than other dyes, allows measurements of challenging cell lines and receptors
- Stringently manufactured, to control and eliminate non-specific assay artifacts

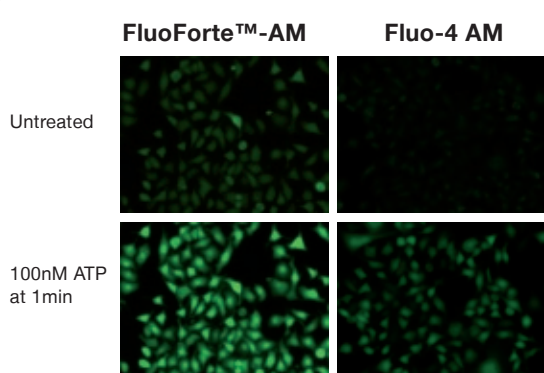


FIGURE 2: HeLa cells were seeded overnight at 40,000 cells per 100 μl per well in a 96-well black wall/clear bottom Corning® Costar® plate. The growth medium was removed, and the cells were incubated with 100 μl of 4 μM Fluo-4 AM or FluoForte™ AM in HHBS at 37°C, 5% CO_2 incubator for 1 hour. The cells were washed twice with 200 μl HHBS, ATP (20 μl /well) was added to achieve concentrations of 100nM with dye efflux inhibitor, then immediately imaged with a fluorescence microscope (Carl Zeiss, Inc.) using FITC channel.

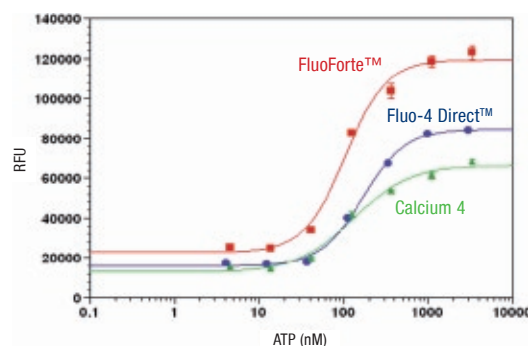


FIGURE 3: ATP dose response curves in CHO-M1 cells, expressing P2Y endogenous receptors. CHO cells were seeded overnight at 40,000 cells per 100 μl per well in a 96-well black wall/clear bottom microplate. The cells were incubated with 100 μl of Life Technologies' Fluo-4 Direct™ kit, Molecular Devices' Calcium 4 kit (both based upon manufacturer's protocol) or Enzo Life Sciences' FluoForte™ dye. ATP (20 μl /well) was added by FlexStation to achieve the final indicated concentrations. No significant difference in EC_{50} of ATP for FluoForte™, Fluo-4 Direct™ and Calcium 4 was observed and FluoForte™ generated the highest intensity signal.

Product	Prod. No.	Size
FluoForte™ Calcium Assay Kit for microplates (starter pack)	ENZ-51017	10 x 96 wells
FluoForte™ Calcium Assay Kit for microplates (high-throughput)	ENZ-51016	100 x 96 wells
FluoForte™ Reagent	ENZ-52014	5 x 50 μg
FluoForte™ Reagent	ENZ-52015	1 mg

incorporating

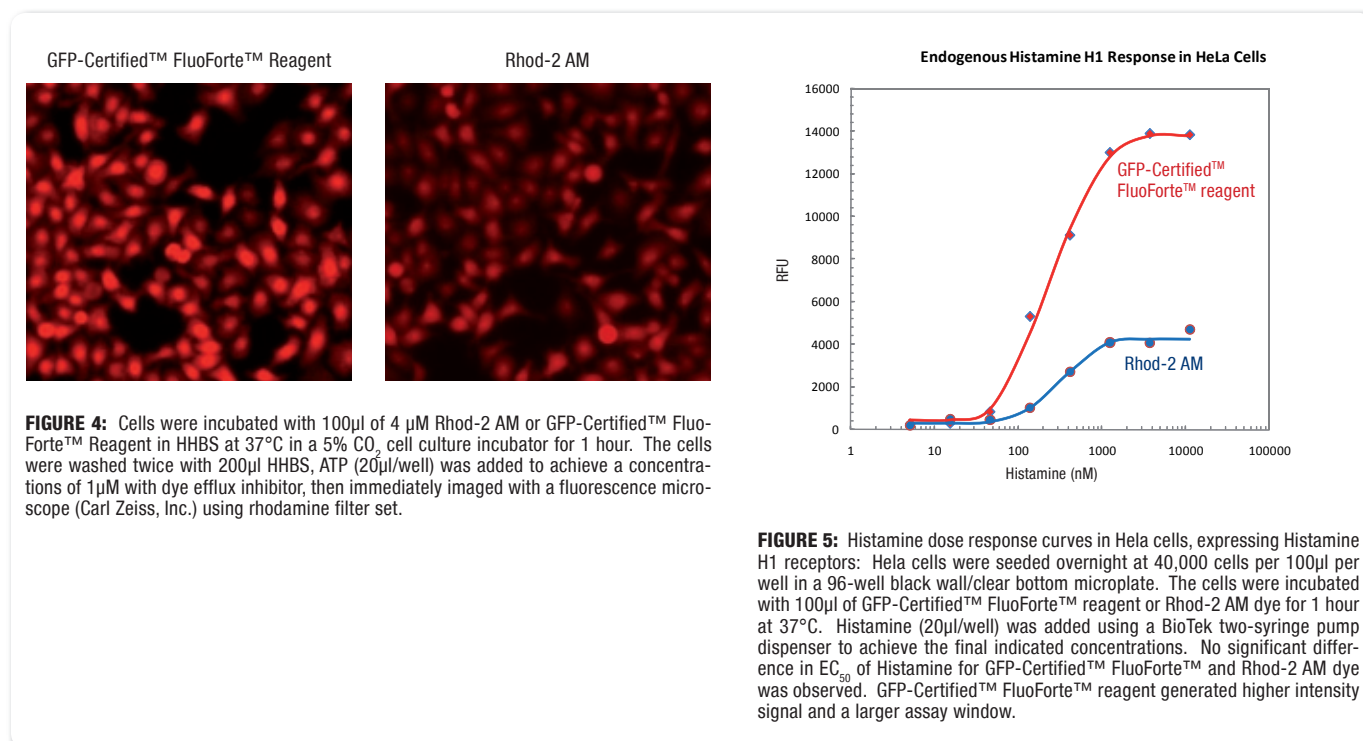
ALEXIS **BIOMOL**
BIOCHEMICALS

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GFP-Certified™ Calcium Assay Kits

Green fluorescent protein (GFP) is a benchmark standard of protein expression and intracellular localization in Biology. Unfortunately, the fluorescent properties of GFP complicate measurement of intracellular calcium mobilization using molecular probes, such as Fluo-3 and Fluo-4, which overlap spectrally with GFP. Fura-2 has been proposed as an alternative for monitoring calcium mobilization in GFP-expressing cells. However, Fura-2 is a UV-excitable dye not readily implemented on common high-throughput imaging plate readers, owing to the high background produced by polystyrene microplates and interference from fluorescent emission of test compounds at this excitation wavelength. Additionally, many microplate readers employ laser sources that limit use to probes excited at visible wavelengths. Rhod-2 is a red-shifted calcium probe, but is relatively dim and tends to concentrate in mitochondria. To address the need for measurement of calcium mobilization in GFP-expressing cells, a new red-shifted molecular probe was devised, providing superior performance compared to Fura-2 and Rhod-2. Our GFP-Certified™ FluoForte™ Calcium Assay Kit, based upon this new probe, is highly efficient, amenable to automation and serves as a robust approach to screening GPCR agonists that mobilize calcium upon activation.

- Designed and manufactured specifically for GFP-expressing cell lines
- The brightest and most sensitive red-shifted fluorescent Ca²⁺ indicator
- GFP-Certified™ FluoForte™ dye (Excitation 530nm, Emission 570nm)



Product	Prod. No.	Size
GFP-Certified™ FluoForte™ Calcium Assay Kit for microplates (starter pack)	ENZ-51020-KP010	10 x 96 wells
GFP-Certified™ FluoForte™ Calcium Assay Kit for microplates (high-throughput)	ENZ-51020-KP100	100 x 96 wells
GFP-Certified™ FluoForte™ Reagent	ENZ-52016-5C50 ENZ-52016-M001	5 x 50 µg 1 mg

Related Products

Product	Prod. No.	Size
Calcein (Ultra Pure)	ENZ-52001	100 mg
Calcein AM (Ultra Pure)	ENZ-52002	1 mg
Calcein Blue (Ultra Pure)	ENZ-52003	250 mg
Fluo-3 AM (Ultra Pure)	ENZ-52004	1 mg
Fluo-3 (Ultra Pure)	ENZ-52005	1 mg
FURA-2 AM (Ultra Pure)	ENZ-52006	1 mg
FURA-2 (Ultra Pure)	ENZ-52007	1 mg
INDO-1 AM (Ultra Pure)	ENZ-52008	1 mg
INDO-1 (Ultra Pure)	ENZ-52009	1 mg
Rhod-2 AM (Ultra Pure)	ENZ-52010	1 mg
Rhod-2 (Ultra Pure)	ENZ-52011	1 mg
Quin-2 AM (Ultra Pure)	ENZ-52012	1 mg
Quin-2 (Ultra Pure)	ENZ-52013	5 mg



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