Quencher Dyes Can Alter the Pharmacokinetic Response Profiles of Living Cells in Fluorescence-based Calcium Mobilization Assays

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ABSTRACT

Fluo4, a calcium sensitive dye, has been a mainstay in calcium mobilization assays for many years. We have developed a new dye, FluoForte™, for increased signal and sensitivity. The FluoForte™ assay kit utilizes a highly efficient FLUORO™ calcium mobilization reagent, which includes a fluorescent dye, a calcium binding buffer, a serum-free control buffer, and a proprietary transport buffer. The FluoForte™ reagent provides better performance in calcium flux assays. These improvements are due to a partially quenched signal, which allows for a more robust assay. Using the FluoForte™ assay kit, we have demonstrated consistently better EC50 and Z’ values. The FluoForte™ reagent provides a higher intensity signal, higher Z’ factor values and larger assay windows than the traditional Fluo-4 reagent. Additionally, FluoForte™ has demonstrated reduced temperature dependencies, improved compatibility with different cell lines and improved assay performance in multiple assay formats.

FluoForte™ Reagent Provides Similar EC50 Values, but Brighter Signal & More Robust Assay Performance than Fluo-4 Dye

Using the Synergy Mx instrument with FluoForte™ assay reagent in the time-resolved Fluo4 format, a degree of fluorescence change is measured using various calcium-sensitive dyes and a fluorescence microplate reader. GPCR assays using fluorescent calcium mobilization reagents, such as Fluo-4, Fluo-4 AM, Fluo-4 AM dye, Fluo-4 AM dye for 1 hour at 37°C. ATP (20 µl/well) was added using a BioTek two syringe pump dispenser to achieve a final concentration of 400 nM. The EC50 values for Fluo-4 AM dye and FluoForte™ reagent are in the range of 100–1000 nM, while the FluoForte™ reagent displays lower temperature dependency, better performance across different cell lines and improved assay windows. The Synergy Mx instrument, in combination with the FluoForte™ Calcium Assay Kit, offers researchers an integrated system to perform fast kinetic, cell-based assays.

FluoForte™ Reagent Displays Less Temperature-dependency for Cell Loading

The FluoForte™ reagent has the following benefits and features:

- Improved signal: FluoForte™ dye is a partially quenched dye, which allows for better signal-to-noise ratio and improved assay performance.
- Reduced temperature dependency: FluoForte™ reagent provides better performance across different cell lines and improved assay windows.
- Improved compatibility: FluoForte™ has demonstrated reduced temperature dependencies, improved compatibility with different cell lines and improved assay performance in multiple assay formats.
- Cost-effective system: The Synergy Mx microplate reader provides a cost-effective system to perform fast kinetic, cell-based assays.

Quencher dyes are employed in calcium mobilization assays in order to avoid removal of serum-containing medium. Exogenous addition of a quencher dye adversely affects receptor agonist interaction.

CONCLUSION

FluoForte™ dye is a partially quenched calcium mobilization reagent that offers several benefits over traditional reagents. It provides better performance across different cell lines and improved assay windows. The Synergy Mx microplate reader provides a cost-effective system to perform fast kinetic, cell-based assays. The FluoForte™ assay kit offers improved signal-to-noise ratio, better performance across different cell lines and improved assay windows. The Synergy Mx instrument, in combination with the FluoForte™ Calcium Assay Kit, offers researchers an integrated system to perform fast kinetic, cell-based assays.