Enzium®

Protease Detection Made Easy
Mix. Read. Innovate.

Fluorescent Protease Activity Assay Technologies: EnSens® vs. FRET/TRF/FLT/AMC

EnSens®	FRET/TRF/FLT/AMC
EnSens is **selective** for a specific protease; can incorporate full recognition site sequence	Substrates are short; not very selective or physiologically relevant
EnSens is recombinant & modular, enabling **rapid development of novel, selective substrates**	Design limits variety of substrates, requires experimentation to develop new substrates
EnSens Far Red Fluorogen (FRF dye) is **resistant to photobleaching** up to 48 hours	The fluorophores can be photobleached more easily than EnSens fluorophore
Long emission wavelength (665nm) reduces interference from other assay components	Many use fluorophores with shorter emission wavelengths more subject to interference
Established **live-cell** protocols for **flow cytometry, standard and 3D cell culture microscopy**	Not easily applicable to cellular or *in vivo* applications
Lower enzyme and substrate concentrations mean more competitive inhibitor hits	Requires higher enzyme and substrate concentrations than EnSens
Substrate and fluorophore are separate, so the fluorophore is constantly renewed in solution	Substrate and fluorophore are one entity—once quenched, it's done
Quickly and directly convert RFU readouts to reaction velocity, k_{cat}, K_m, IC_{50}, etc.	Can involve complicated algorithms and data manipulation
Standard plate or cuvette fluorimeter is sufficient	Can involve complicated/expensive equipment
High signal-to-noise ratios: 6-30X	Higher background than EnSens
No energy transfer involved, minimum self-quenching	Free fluorophore can mask energy transfer (self-quenching)
Substrate and dye are **highly soluble**	Low aqueous solubility
Substrate is an scFv-based protein, which confers **high stability** *in vitro* and *in vivo*	Limited stability *in vitro* and *in vivo*
Stable in wide range of pH, salt, & solvent conditions	Can be pH sensitive

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