

IRDYE® 800RS CASEIN PROTEASE SUBSTRATE

IRDye® 800RS Casein Protease Substrate is a general substrate used to detect protease activity. Casein, a milk protein, is over-labeled with IRDye 800RS to produce a quenched substrate for use in protease assays. This near-infrared protease substrate has significant advantages compared to protease assays using visible fluorescence.

Advantages:

- Simple – Incubate sample or known protease with IRDye 800RS Casein Protease Substrate, measure fluorescence, identify samples containing protease, and if needed, quantify protease concentration based on a standard curve.
- Enhanced assay sensitivity due to low background fluorescence at near-infrared wavelengths.
- Improved detection limits of protease activity.
- Requires a fraction of the substrate compared to visible fluorescence kits.
- Up to 20-fold fluorescence intensity enhancement upon digestion.
- Can be used for protease inhibitor screening.
- Versatile – No stop solution to add and fluorescence is detected at the end of the incubation. System allows for monitoring reactions over time.

Assay Description

When casein is over-labeled with IRDye 800RS, the fluorophores are quenched due to intramolecular interactions of the dye molecules (Fig. 1).

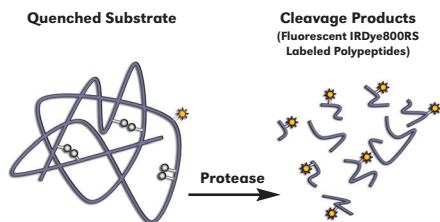


Figure 1. Schematic of IRDye 800RS Casein Protease Substrate. Fluorescence increases upon protease substrate digestion.

The assay is initiated by adding a protease of your choice to the IRDye 800RS Casein Protease Substrate, incubated and subsequently read with the Odyssey® or Aeries® Infrared Imaging Systems. Digestion of the IRDye 800RS Casein Protease Substrate releases IRDye labeled peptides that fluoresce. Upon excitation, the protease activity can be determined by measuring the near-infrared fluorescence emission. Near-infrared (NIR) dyes provide a unique detection environment due to low background fluorescence from biological samples and microplates.

Assay Performance

Protease Detection. The performance of this NIR protease assay is demonstrated by digesting the IRDye 800RS Casein Protease Substrate with trypsin. The fluorescence intensity significantly increases (Figure 2) and correlates to the enzyme concentration.

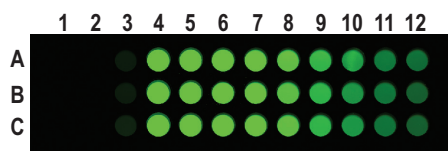


Figure 2. A, B, and C are three replicates. Column 1: buffer only; column 2: trypsin only; and column 3: the substrate only, IRDye 800RS Casein. A two-fold dilution series of trypsin from 5 µg/mL to 19.7 ng/mL is shown in columns 4-12 respectively, at a substrate concentration of 20 nM.

When IRDye 800RS fluorescence intensity is plotted against trypsin concentration (Figure 3), the increase in fluorescence is consistently up to 20-fold, compared to only 5-fold enhancement from other commercial assays. Figure 3 also demonstrates the robustness of the assay, which is unaffected by the presence of materials that fluoresce at visible wavelengths. Fluorescein was added to the assay to simulate the fluorescence from biological compounds. The fluorescence response in the presence

of fluorescein is identical to the response in the absence of fluorescein, showing the ability of the near-infrared system to reject materials that absorb in visible wavelengths.

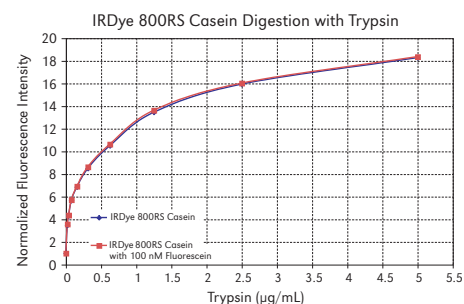


Figure 3. Normalized fluorescence intensity readings on an Aeries® Automated Imaging System (LI-COR Biosciences) for IRDye 800RS Casein reactions incubated with trypsin in the presence or absence of 100 nM fluorescein.

Sensitivity. Figure 4 shows the integrated fluorescence intensity linear response with sub-nanogram amounts of trypsin. The data in Table 1 show a 2-30 fold improvement in the limit of detection (LOD) for three unique proteases with IRDye 800RS Casein. This assay only requires 1/10 the amount of labeled substrate and offers better detection limits than typical visible fluorescence-based protease assays.

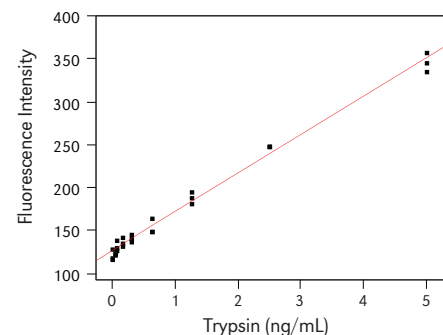


Figure 4. The integrated intensity of IRDye 800RS Casein fluorescence on Aeries in response to the trypsin amounts when the enzyme is in the range of a few nanograms.

Table 1. Limit of detection comparisons for IRDye 800RS Casein and BODIPY® Casein.

Enzyme (Source)	Class	IRDye 800RS Casein 20% Above Blank LOD ^a (μUnits)	BODIPY Casein 10-20% Above Blank LOD ^b (μUnits)	IRDye 800RS Casein 'Rigorous' LOD ^c (μUnits)
Trypsin (porcine pancreas)	Serine Protease	820	13,000	2700
Thermolysin (<i>B. proteolyticus rokko</i>)	Acid Protease	6.8	44	19
Papain (papaya latex)	Sulfhydryl Protease	6.2	210	6.1

^aLimit of detection is defined as the enzyme amount required to cause a 20% change in fluorescence signal compared to the control samples.

^bMolecular Probes EnzChek™ Protease Assay Kit product information. Limit of detection defined as the amount of enzyme required to cause a 10-20% change in fluorescence compared to the control sample at 22 °C.

^cA more rigorous definition of limit of detection is defined by the amount of enzyme required to observe fluorescence signal that is above the minimal response (MR) detection level. This is defined by the following equation: $\text{Detection Level}_{\text{MR}} = \mu_{\text{MR}} + 3\sigma_{\text{MR}}$ where μ_{MR} is the mean of the minimal response and σ_{MR} is the standard deviation of the minimal response.

Protease Inhibition. IRDye 800RS

Casein Protease Substrate also may be used in studies of protease inhibition and inhibitor screening. Aprotinin, a known trypsin inhibitor (figure 5), was used with IRDye 800RS Casein to detect trypsin inhibition. The fluorescence intensity change between total trypsin activity suppression (at 10 μg/mL aprotinin) and no inhibition is more than 20-fold at 100% substrate conversion. In a similar assay with BODIPY-casein, the dynamic range is only 2-fold. Low background fluorescence interference at NIR wavelengths allows sensitive detection of enzyme activity and its effect by inhibitors, which otherwise would be difficult with visible fluorescence probes.

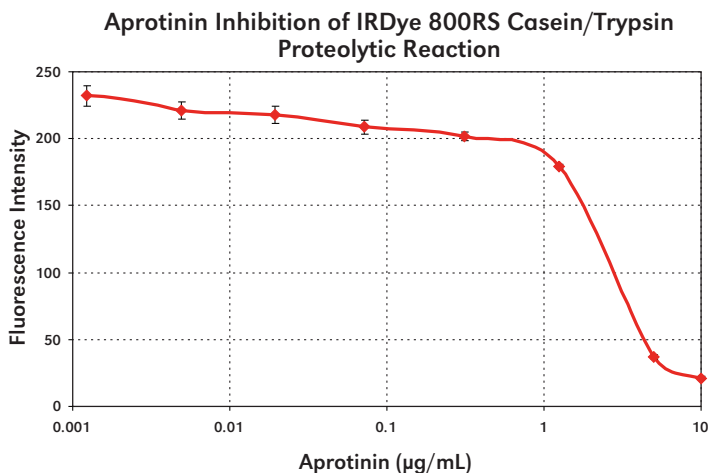


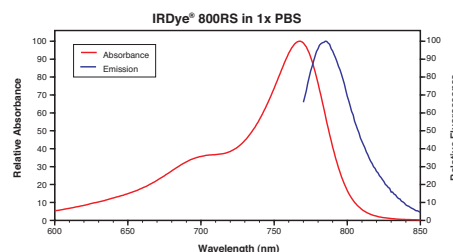
Figure 5. Aprotinin inhibition of IRDye 800RS Casein digestion with trypsin.

Specifications

Each IRDye 800RS Casein Protease Substrate kit is sufficient for 1000 assays (ten 96-well plates).

Kit Contents:

- 4 tubes of lyophilized IRDye 800RS Casein Protease Substrate, (6.3 nmol per tube).
- 0.5% Sodium Azide (100 μl)
- Protocol



Solvent	Ext. Coeff. (M ⁻¹ cm ⁻¹)	Abs Max (nm)	Em Max (nm)	MW (g/mole)
Methanol	300,000	770	786	961
Water	200,000	767	786	961
1X PBS	200,000	767	786	961
PBS: Methanol	270,000	770	786	961

Ordering Information

IRDye 800RS Casein926-08587
Protease Substrate Kit

IRDye 800RS Casein926-09116
Protease Substrate
(Trial Size)

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