Pro3C HRV-3C Protease FRET activity assay kit

Description:

Product number: 2808

Number of reaction: 10 protease samples + 2 calibration curves

Application: Assay for precise HRV-3C protease activity measurement using protein substrate based on FRET. For comparison of different lots and types (mutants) of 3C protease with our standard HRV-3C protease.

Introduction:

The Pro3C HRV-3C protease fluorescent activity assay uses protein substrate based on fluorescent energy transfer (FRET). The principle of FRET relies on energy transfer of excitation energy of a donor fluorophore to a nearby acceptor fluorophore. The 54 kDa FRET substrate protein is composed by two fluorescent proteins, green 26 kDa and red 28 kDa proteins linked with HRV-3C protease recognition sequence LEVLFQ|GP. The substrate is specifically cleaved to fluorescent monomers, which results in quantitative decrease of fluorescent intensity at 580-650 nm (emission range, the excitation range is 490-515 nm). The Pro3C HRV-3C protease fluorescent activity assay is suitable for precise protease activity measurements, monitoring or high-throughput screening of 3C protease variants, substrate specificity measurements, etc. All components are manufactured in certified laboratory environment and could be used in GMP certified downstream processes. Recombinant proteins (3C-protease and FRET 3C protease substrate) are purified by affinity chromatography, size exclusion chromatography and desalting.

Precautions and Disclaimer:

This kit is manufactured under ISO 9001 and ISO 13485. It is not intended to use for a direct clinical diagnostic use. Country of origin: Czech Republic. The kit does not contain animal products.

Components:

- FRET 3C protease substrate (0,5 mg/ml)
- reference Pro3C HRV-3C protease (800 U/ml)
- Protease reaction buffer (PRB)
- DTT (1M DTT)
- ultra pure water

Storage:

-20˚C (for several days 4˚C)

Example Protease Assay Procedure:

IN SEPARATE TUBES:

1. Determine the desired number of reactions (NOTE: always add blank reaction – without protease).
2. Let the reagents thaw on ice properly. **Leave on ice during all preparations.**
3. Prepare mixture for all reactions together to eliminate pipetting errors.
   For 1 reaction (final volume 375 µl):
   - 138 µl ultra pure H_2O
   - 200 µl FRET 3C protease substrate (0,5 mg/ml)
   - 37,5 µl Protease reaction buffer
   - 0,375 µl 1M DTT
4. Prepare calibration curve.
   It is suitable to choose your highest point of calibration as a mixture for 1 reaction (final amount of FRET in reaction is 100 µg). We recommend to prepare minimally 6 points calibration plus zero.

Table 1: Example of calibration curve and its preparation:

m_{FRET} (µg)	V_{FRET} (µl)	V_{H2O} (µl)	V_{PRB} (µl)
0	0	337,5	37,5
3	6,1	331,4	37,5
6	12,5	325,0	37,5

12	25,0	312,5	37,5
25	50,0	287,5	37,5
50	100,0	237,5	37,5
100	200,0	137,5	37,5

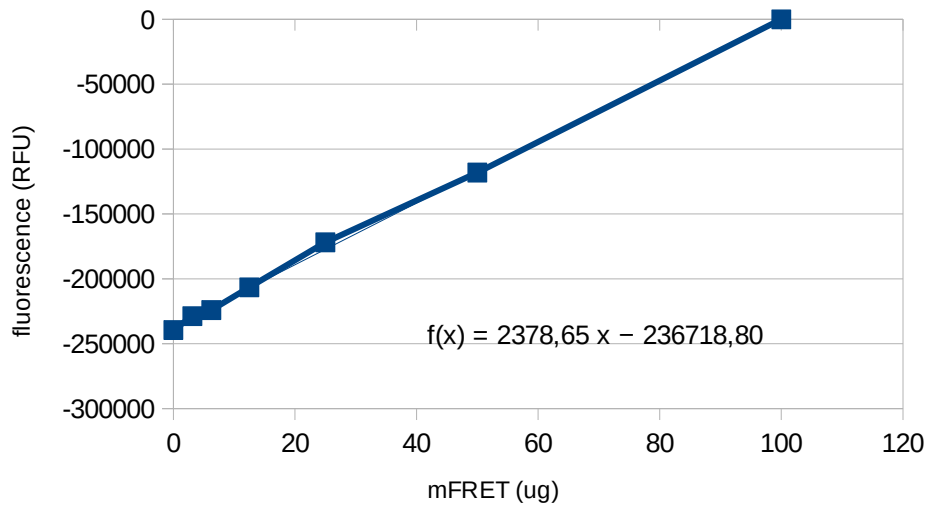
5. We recommend to dilute protease samples 10x to slower the digest reaction and to gain precise results. To 375 µl reaction mixture add 5 µl of protease (for the **blank reaction** use water). Measure fluorescence immediately at excitation wavelength 490-515 nm. Incubate at 30˚C or 6 ˚C according to your needs. Measure repeatedly every hour or as desired.
   For the **correction of the maturation** of the FRET substrate, measure the **blank** each time point of the reaction.
   Activity of proteases should decrease during 3 hours approximately, depending on the activity of your samples.

Data evaluation:

1. Emission wavelength range is 580-650 nm. In case your fluorometer has more channels with different wavelength range, make sure you are using the correct one. (**Excitation and emission wavelength ranges differ!**)

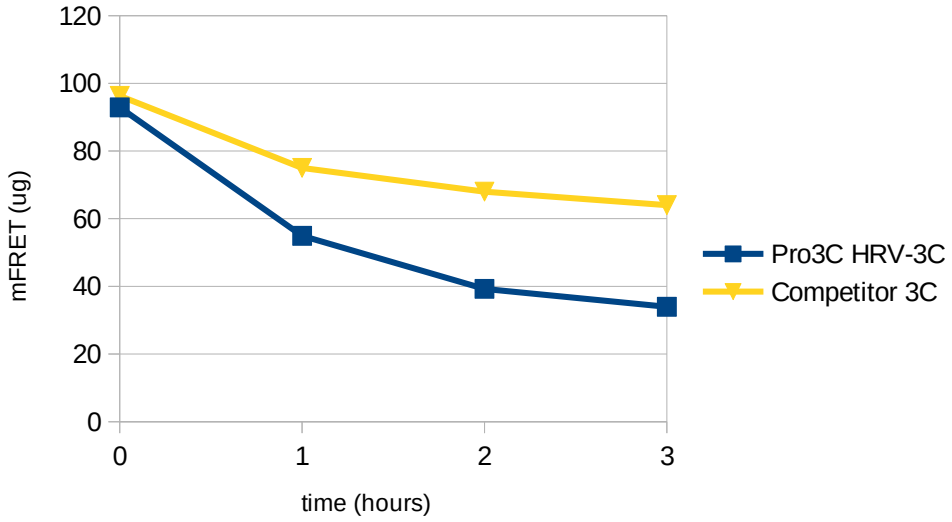
2. **Maturation correction:** subtract blank sample for each measurement from protease sample of corresponding time point. This step serves as correction of increasing fluorescence in time. Amount of FRET is decreasing with time, but the fluorescence of the not digested FRET substrate is increasing in time due to substrate maturation – this effect is visible at data of blank sample, which are used for the fluorescence correction (FL_{COR}).

3. **Calibration curve.** Subtract measured fluorescence of a sample with highest concentration (blank sample for calibration) from all points of calibration. Plot a graph against amounts of FRET. Add a linear trend line and show the equation of trend line **(y=A·x+B)**. You will get constants A and B, in this equation **y=measured fluorescence** and **x=amount of FRET**. Apply this equation on your measured data and you will get precise amounts of FRET for your sample for each hour.



Graph 1: Example graph of the calibration curve. Fluorescence of calibration points is calculated with the respect to the amount of FRET as a decrease in fluorescence, subtracting the signal of 100ug of FRET (highest point of calibration) from each calibration point. The 0 point is set to the amount of FRET added to the reaction at the beginning of measurement (100ug), where no substrate is digested yet. The formula is calculated from the trend line approximation using a spread sheet application of choice.

4. Graphical representation of results. The corrected fluorescence (FL_{COR}) from the point 2. is used for the calculation of the non-digested amount of FRET substrate ($FRET_{ND}$) at each time point by this equation: $FRET_{ND}=(FL_{COR}-B)/A$. Plot results into a line graph for an interpretation of specific activity of measured proteases.



Graph 2: Example graph of decreasing amount of FRET (ug) against time (hours) in measured samples. Fluorescence is decreasing into negative numbers after subtracting blank sample. The start (0 hours) correspond to 0 fluorescence upon subtraction of the background.

5. The **specific activity** of tested protease is calculated from the amount of digested FRET substrate by the following equation: 5ul of 10x diluted reference HRV-3C Protease (originally 800U/ml), equals 0.4U in the reaction, digests 45ug of FRET substrate (undigested FRET subtracted from 100ug of input) in 1h at 6°C. Therefore, 1U of reference HRV-3C digests 112.5ug of FRET substrate in 1h at 6°C.