

Protease Detection Kit

Contamination Control Kits

Cat.-No.	Amount
PP-404S	50 reactions
PP-404L	200 reactions

For *in vitro* use only

Quality guaranteed for 12 months.

Store Substrate Solution and Positive Control at -20°C, Incubation Buffer, Assay Buffer and Precipitation Reagent at -20 to 4°C. Aliquoting of reagents and handling in the dark is recommended. Protect Substrate Solution from light!

Multiple freeze-thaw cycles enhance background fluorescence.

Kit contents

Substrate Solution (violet cap)

S pack: 2.6 ml

L pack: 10.4 ml

Incubation Buffer (green cap)

S pack: 3 ml

L pack: 12 ml

Assay Buffer (yellow cap)

S pack: 30 ml

L pack: 125 ml

Precipitation Reagent (red cap)

S pack: 30 ml

L pack: 125 ml

Positive Control (blue cap)

S pack: 2.6 ml

L pack: 10.4 ml

Additionally required material

- spectrophotometer or fluorometer
- tubes, cuvettes or microtiter plates

- thermo block or incubator at 37°C
- pipettes and filter tips
- micro centrifuge

Description

This Protease Detection Kit detects a wide variety of proteases, including serine proteases, cysteine proteases and acid proteases in biological samples. The kit is based on a labeled casein derivative as protease substrate, to be incubated with the sample for 2 to 20 hours at 37°C. Active protease present cleaves labeled casein into peptides. Precipitation Reagent is added to reaction mix. Undigested casein precipitates whereas cleaved peptides stay in solution and are measured after centrifugation in the supernatant at 492 nm. The intensity of absorbance is proportional to the total protease activity in the sample.

The pH of the Incubation Buffer is suitable for most physiological applications. If you however, a specific work with enzyme with a unique pH optimum preparing of a pH-specific buffer may be useful.

The detection limit of the assay is approximately 10 ng/sample of protease when a long incubation time is used. A long incubation time is not advised generally, because of auto degradation and thermal breakdown. You may enhance the sensitivity by using a fluorometer (excitation 490 nm and emission 525 nm) instead of a spectrophotometer (absorbance at 492 nm) to identify the cleaved peptides. This is not possible for samples with high fluorescent background.

Due to high sensitivity of the kit, please pay special attention to avoid cross contaminations.

Protocol

Cuvette operation:

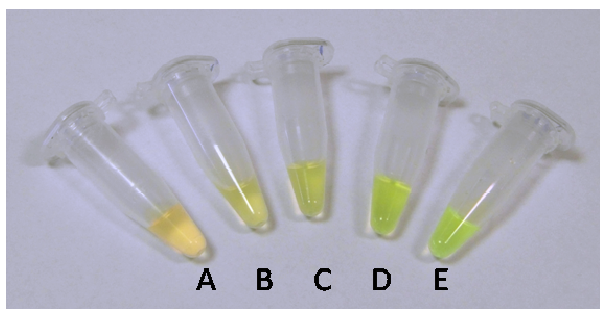
- unit substances given below in a 1.5 ml tube

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	Sample	Blank
Substrate Solution	50 μ l	50 μ l
Incubation Buffer	50 μ l	50 μ l
sterile water	-	100 μ l
Sample	100 μ l	-

- incubate at 37°C for 2 h to 20 h



After incubation color of sample already allows a visual estimate of protease activity.

A: water control

B: weakly protease activity

C: medium protease activity

D: high protease activity

E: Positive Control

- quench reaction by addition of 500 μ l Precipitation Reagent
- mix shortly and incubate the tubes at room temperature for 30 min
- centrifuge at 12.000 g for 5 min
- convert 400 μ l of supernatant to another microfuge tube
- add 600 μ l of Assay Buffer and mix
- read absorbance at 492 nm versus blank

Microtiter plate operation

- unit substances given below in each well of a 96 well round bottom-plate. Instead of this, you may use 0.5 ml tubes.

	Sample	Blank
Substrate Solution	25 μ l	25 μ l
Incubation Buffer	25 μ l	25 μ l
sterile water	-	25 μ l
Sample	25 μ l	-

- mix and incubate at 37°C for 2 h to 20 h in a wet chamber
- add 120 μ l Precipitation Reagent to each well; incubate at 37°C for 30 min, or 2 h by room temperature in a wet chamber
- centrifuge the plate for 15 min at 500 g at 4°C, the tubes at 12.000 g for 5 min
- take 50 μ l supernatant and transfer to a flat-bottom 96 well plate; use a black one - if you want to measure fluorescent, a clear one - if you want to measure absorbance
- add 150 μ l Assay Buffer to each well and mix
- read absorbance for each well at 492 nm using a plate spectrophotometer, or excitation at 490 nm and emission at 525 nm on a fluorometer

Interpretation

- subtract blank from measured values
- Positive Control typically results in an $A_{492\text{ nm}} > 0.1$
- measured values > 0.02 at $A_{492\text{ nm}}$ are indicators for protease activity present in the sample

References

Twining, S. S. (1984). Casein assay for proteolytic enzymes. *Anal. Biochem.* 143:30–34.