

## Capture proteasome activity assay (CAPA)

### KEYWORDS

- Proteasome subtypes
- Proteasome Activity assay
- Fluorogenic peptides
- Proteasome inhibitors

### Technology Market :

#### Measurement of proteasome subtypes activity

Four cell lines were developed, each expressing one specific subtype of proteasome:

- standard proteasome (SP)
- immunoproteasome (IP),
- single intermediate proteasome  $\beta 5i$  (SIP),
- double intermediate proteasome  $\beta 1i$ - $\beta 5i$  (DIP).

These cell lines were characterized using proprietary antibodies developed against the native subunits of proteasome<sup>1</sup> and will be provided as cell lysates.

The CAPA test consists in capturing a specific proteasome subtype from the cell lysate on antibody-coated plates and using classic fluorogenic substrates for direct proteasome activity read-out.

### Features

- Choice of four different cell types each expressing a different proteasome subtype.
- Protocol to measure proteasome activities based on a single step of proteasome antibody capture.

### Applications

- Measurement of the activity of the different proteasome subtypes
- Useful for the identification of proteasome subtype-specific inhibitors

### Advantages

- Fast and easy ELISA-based technology
- Bypasses the heavy and complex procedures of proteasome purification
- The step of proteasome capture eliminates the need of using proteases or proteasome inhibitors for result interpretation
- Provides a direct read-out of the activity of different proteasome subtypes

### References

1. Guillaume, et al. "Two abundant proteasome subtypes that uniquely process some antigens presented by HLA class I molecules," *Proc. Natl. Acad. Sci. USA*, vol. 107, pp. 18599-18604, 2010.
2. Vigneron, et al. "The capture proteasome assay: A method to measure proteasome activity in vitro", *Analytical Biochemistry* 482 (2015) 7-15.

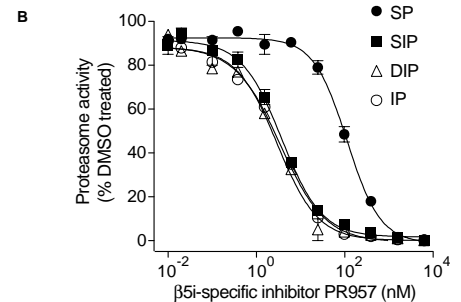
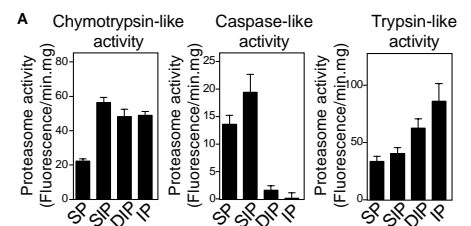
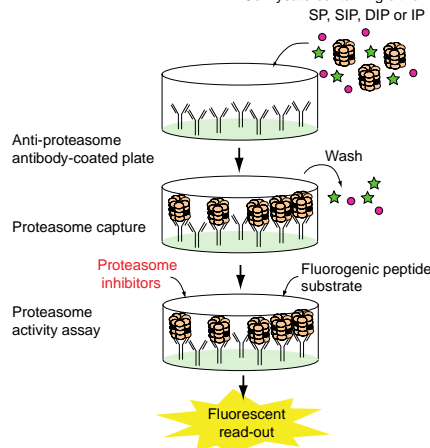


Fig. 2. Measurement of the activity of proteasome captured from 293 cells expressing either SP, IP, SIP or DIP. Captured proteasome are incubated in the presence of commercially available fluorogenic substrates to measure their chymotrypsin, caspase and trypsin-like activities, respectively (A). The chymotrypsin-like activities of each proteasome type was measured in the presence of increasing concentration of the  $\beta 5i$ -specific proteasome inhibitor PR957. The  $\beta 5i$  subunit is present in IP, SIP and DIP, but not in SP (B).