Kex2 Protease (Lys/Arg-Arg), recombinant

Product Information Sheet
 # EP0410/EP0450

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SUMMARY

shipped on dry ice; store at -80 °C

For research use only

Product Description and Application

- specific serine endoproteinase
- cleaves amino acid sequence N-Arg-Arg/-C and N-Lys-Arg/-C at the carboxyl end
- suited for protein sequencing and cleavage of fusion proteins with an appropriate recognition sequence

In the series of endoproteinases we also offer the newly developed endoproteinase Lys/Arg-Arg. The specific serine endoproteinase (universal serine-protease) has a MW of 68 kDa. It cleaves at the carboxyl end of the recognition sequences: Arg-Arg/X and Lys-Arg/X and thus provides new possibilities for e.g. the development of fusion protein systems with alternative cleavage sites.

Technical Details

Source:	*Saccharomyces cerevisiae*
Activators: pH-	Endoproteinase Lys/Arg-Arg is strongly Ca^{2+} dependent and has a optimum at pH 7.0
Inhibitors:	Ala-Lys-Arg-chloro-methyl ketone **Note:** *Endoproteinase Lys/Arg-Arg is not inhibited by phenyl-methyl-sulfonyl-fluoride and tosyl-lysine-chloro-methylketone.*
Reaction buffer:	50 mM Tris-HCl or HEPES, 5 mM $CaCl_2$, pH 7.0 (0.5 mM PMSF; 0.1% Triton X-100 is not included, but might be added if required by your application). For fusion proteins the reaction conditions have to be determined empirically.
Substrate solution:	Benzyloxycarbonyl-L-tyrosyl-L-lysyl-L-arginin-4-nitroanilid (Z-L-Tyr-Lys-Arg-pNA ◦ TFA salt, Bachem No. L1250). The substrate is dissolved in reaction buffer (OD_{315} = 12). This solution can be stored at -20 °C for several months. Thaw at room temperature immediately before use.
Unit definition:	1 unit endoproteinase Lys/Arg-Arg releases 1 µmole 4-nitroaniline per minute in reaction buffer at pH 7.0 at RT.
Specific activity:	2.2 U/ml

Assays:

The substrate solution (OD_{315} = 12) was thawed at RT. Assay solution: 100 µl of the substrate solution was mixed with 400 µl reaction buffer and put into a 500 µl cuvette (1 cm optical path length). For this solution the photometer was adjusted to 0.0 and a range from 0 to 0.5 OD_{405} was chosen. The assay solution was mixed with 10 µl of a 1:10 dilution of the endoproteinase Lys/Arg-Arg. The optical density was measured at 405 nm for 5 minutes (at RT). The initial slope was plotted as a straight line and, after 1 minute, the value of the straight line was read (Δ OD_{405}, for 1 minute).

$$\frac{\Delta\ OD_{405} \times 1\ min \times 100 \times 10}{\varepsilon \times 2} = \text{activity in units/min x ml (undiluted)}$$

(Note: ε = 9.94)

References

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Order Information, Shipping and Storage

Order#	Product	Quantity
EP0410	Kex2 Protease (Lys/Arg-Arg), recombinant	10 U
EP0450	Kex2 Protease (Lys/Arg-Arg), recombinant	50 U
shipped on dry ice; store at -80 °C		