Recombinant Human HTRA2/Omi

Catalog Number: 1458-HT

DESCRIPTION

Source	*E. coli*-derived Ala134-Glu458, with a C-terminal 6-His tag Accession # O43464
N-terminal Sequence Analysis	Ala134
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	38 kDa, reducing conditions
Activity	Measured by its ability to cleave β-casein. >95% cleavage of β-casein, as measured under the described conditions.
Purity	>85%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 8.0
- Recombinant Human HTRA2/Omi (rhHTRA2) (Catalog # 1458-HT)
- Substrate: β-Casein (Sigma, Catalog # C-6905), 1.0 mg/mL stock in 25 mM Tris, 0.15 M NaCl, pH 7.5
- SDS-PAGE and silver staining reagents

Assay

1. Dilute Substrate to 0.4 mg/mL in Assay Buffer.
2. Dilute rhHTRA2 to 0.04 mg/mL in Assay Buffer.
3. Add 25 µL of substrate and 25 µL of rhHTRA2 to reaction tube. Include a control with 25 µL Substrate and 25 µL Assay Buffer.
4. Incubate the reaction and control for 1 hour at 45 °C.
5. Stop the reaction by adding SDS-PAGE sample buffer.
6. Analyze the cleavage by SDS-PAGE followed by silver staining.

Final Assay Conditions

Per Reaction:

- rhHTRA2: 0.02 mg/mL
- Substrate: 0.2 mg/mL

PREPARATION AND STORAGE

Shipping

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

HtrA2/Omi is the mammalian homologue of bacterial high temperature requirement protein (HtrA). HtrA2/Omi localizes to the mitochondria and is processed to expose an amino-terminal Reaper-like motif similar to SMAC/Diablo. HtrA2/Omi is released from the mitochondria in response to apoptotic insult and can interact with the BIR2 or BIR3 domains of XIAP to relieve caspase-IAP inhibition. This effect can be measured by reversing XIAP-BIR2 (Catalog # 786-XB) inhibition of Caspase-7 (Catalog # 823-C7) cleavage of a fluorogenic peptide (DEVD-AFC, MP Bio, Catalog # AFC-138). IC_{50} values for this effect are typically between 0.2 and 1.5 µM. HtrA2/Omi is trimeric and functions as a serine protease. The serine protease activity may play a more central role in apoptosis than its IAP antagonizing function. A PDZ domain regulates the serine protease activity by blocking access to the active site. The specificity of the protease is yet to be defined and no endogenous substrates are known to date.

References:

1. Suzuki, Y. *et al.* (2001) Mol. Cell. **8**:613.
2. van Loo, G. *et al.* (2002) Cell Death & Diff. **9**:20.
3. Hedge, R. *et al.* (2001) J. Biol. Chem. **277**:432.
4. Verhagen, A. *et al.* (2001) J. Biol. Chem. **277**:445.
5. Martins, L. *et al.* (2002) J. Biol. Chem. **277**:439.
6. Silke, J., and A. Verhagen (2002) Cell Death & Diff. **9**:362.
7. Savopoulos, J. *et al.* (2000) Protein Expression & Purification **19**:227.

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449