Recombinant Human TMPRSS2 His-tag

Catalog Number: 11457-TP

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human TMPRSS2 protein Trp106-Gly492, with modifications in the non-catalytic chain and a C-terminal 6-His tag Accession # NP_005647.3
N-terminal Sequence Analysis	Trp106 & Phe108 (non-catalytic chain), Arg255 & Ile256 (catalytic chain)
Predicted Molecular Mass	17 kDa (non-catalytic), 29 kDa (catalytic)

SPECIFICATIONS

SDS-PAGE	19-25 kDa (non-catalytic) & 25-33 kDa (catalytic) under reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Boc-QAR-AMC (Catalog # ES014). The specific activity is >7000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 50 mM NaCl, 0.01% Tween-20, pH 9.0
- Recombinant Human (rhTMPRSS2) TMPRSS2 His-tag (Catalog # 11457-TP)
- Substrate: BOC-Gln-Ala-Arg-AMC (Catalog # ES014), 10 mM stock in DMSO
- Black 96 well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhTMPRSS2 to 0.1 µg/mL in Assay Buffer.
2. Dilute Substrate to 400 µM in Assay Buffer.
3. Load into a plate 50 µL of 0.1 µg/mL rhTMPRSS2, and start the reaction by adding 50 µL of 400 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 400 µM Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC)

Final Assay Conditions

Per Well:

- rhTMPRSS2: 0.005 µg
- Substrate: 200 µM

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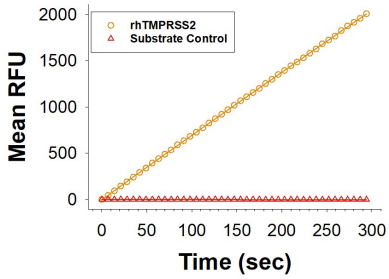
PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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.

DATA

Enzyme Activity



Recombinant Human TMPRSS2 His-tag Protein Enzyme Activity. Recombinant Human TMPRSS2 His-tag Protein (Catalog # 11457-TP) is measured by its ability to cleave the fluorogenic peptide substrate Boc-QAR-AMC (Catalog # ES014).

BACKGROUND

Recombinant Transmembrane protease serine 2 (TMPRSS2), also referred to as serine protease 10 (PRSS10), is a member of the type II transmembrane serine protease (TTSP) peptidase S1 family (1). Similar to other members of the TTSP family, TMPRSS2 is produced as a zymogen and undergoes post-transcriptional modifications to allow proteolytic autoactivation into a non-catalytic chain, composed of an LDLR-A and SRCR domain, and a catalytic chain with a highly conserved serine protease (SP) domain. The TMPRSS2 SP domain uniquely contains an unpaired cysteine bordered by a hydrophobic patch thought to accommodate various binding partners (2). TMPRSS2 consists of three distinct regions, including an intracellular domain, a single-pass transmembrane domain, and an extracellular domain and can be membrane bound or found in a soluble secreted form (1, 2). The TMPRSS2 gene harbors androgen-responsive elements and expressed through the stimulation of the AR (1) in tissues with epithelial cells at high levels in prostate and relatively lower levels of expression in lungs, colon, liver, kidneys and pancreas (2-4). TMPRSS2 activates protease activated receptor 2 (PAR-2), a G-protein coupled receptor, causing the upregulation of matrix metalloproteinase-2 and -9, key proteases in the metastasis of tumor cells (5) and in prostate cancer has been shown to activate pro-hepatocyte growth factor resulting in subsequent c-MET signaling known to regulate the tumor microenvironment, immune infiltration, and immune response (6,7). TMPRSS2 expression may be involved broadly in cancer prognosis through this pathway as reported in lung adenocarcinoma and breast invasive carcinoma (7). In addition to playing a role in cancer, TMPRSS2 is the host protease determined to be responsible for processing of viral protein to facilitate host entry in several viruses including spike protein in SARS-CoV-2, SARS-CoV, and MERS-CoV and hemagglutinin in influenza A viruses (3,4,8-11). TMPRSS2 represents a prime target for therapeutic intervention in aggressive cancers and to block initial viral influenza and coronavirus infection (2, 3, 11).

References:

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