

APJ365Hu01 100µg

Active Active Catechol-O-Methyltransferase (COMT)

Organism Species: *Homo sapiens* (Human)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Gly52~Pro271

Tags: N-terminal His-tag

Purity: >95%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 5.6

Predicted Molecular Mass: 25.6kDa

Accurate Molecular Mass: 26kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

GDTKEQRIL NHVLQHAEPG NAQSVLEAID TYCEQKEWAM NVGDKKGGKIV
DAVIQEHQPS VLLELGAYCG YSAVRMARLL SPGARLITIE INPDCAAITQ
RMVDFAGVKD KVTLVVGASQ DIIPQLKKKY DVDTLDMVFL DHWKDRYLPD
TLLLEECGLL RKGTVLLADN VICPGAPDFL AHVRGSSCFE CTHYQSFLEY
REVVDGLEKA IYKGGPSEAG P

[ACTIVITY]

Catechol-O-Methyltransferase (COMT) is one of several enzymes that degrade catecholamines (such as dopamine, epinephrine, and norepinephrine), catecholestrogens, and various drugs and substances having a catechol structure. Two isoforms of COMT are produced: the soluble short form (S-COMT) and the membrane bound long form (MB-COMT). COMT is involved in the inactivation of the catecholamine neurotransmitters (dopamine, epinephrine, and norepinephrine). The enzyme introduces a methyl group to the catecholamine, which is donated by S-adenosyl methionine (SAM). Any compound having a catechol structure, like catecholestrogens and catechol-containing flavonoids, are substrates of COMT. Besides, UDP glucuronosyltransferase 2 family, polypeptide B1 (UGT2B1) has been identified as an interactor of COMT, thus a binding ELISA assay was conducted to detect the interaction of recombinant human COMT and recombinant human UGT2B1. Briefly, COMT were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to UGT2B1-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-COMT pAb, then aspirated and washed

3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C . Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of COMT and UGT2B1 was shown in Figure 1, and this effect was in a dose dependent manner.

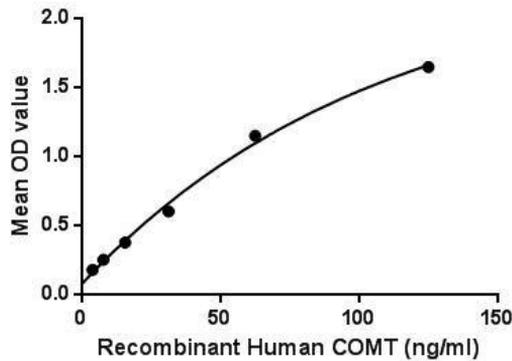


Figure 1. The binding activity of COMT with UGT2B1.

[IDENTIFICATION]

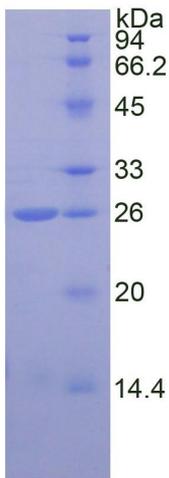


Figure 2. SDS-PAGE

Sample: Active recombinant COMT, Human

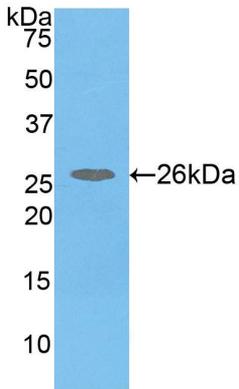


Figure 3. Western Blot

Sample: Recombinant COMT, Human;

Antibody: Rabbit Anti-Human COMT Ab (PAJ365Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.