Recombinant Human Aldo-keto Reductase 1C3/AKR1C3

Catalog Number: 7678-DH

DESCRIPTION

Source	*E. coli*-derived Asp2-Tyr323, with an N-terminal Met and 6-His tag Accession # P42330
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	36-37 kDa, reducing conditions
Activity	Measured by its ability to catalyze the reduction of phenanthrenequinone. The specific activity is >115 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Brij and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 100 mM Sodium Phosphate, pH 6.0
- Recombinant Human Aldo-keto Reductase 1C3/AKR1C3 (rhAKR1C3) (Catalog # 7678-DH)
- 9,10-Phenanthrenequinone (PQ) (Sigma, Catalog # 156507), 5 mM stock in N,N-Dimethylformamide
- β-Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (β-NADPH) (Sigma, Catalog # N7505), 10 mM in deionized water
- UV Plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhAKR1C3 to 20 µg/mL in Assay Buffer.
2. Prepare a Reaction Mixture containing 40 µM PQ and 400 µM β-NADPH in Assay Buffer.
3. In a plate, load 50 µL of 20 µg/mL rhAKR1C3, and start the reaction by adding 50 µL of Reaction Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Reaction Mixture.
4. Read at an absorbance of 340 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x -1 x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 6270 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhAKR1C3: 1 µg
- PQ: 20 µM
- β-NADPH: 200 µM

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

AKR1C3 is a member of the aldo-keto reductase (AKR) superfamily. It catalyzes oxidation/reduction reactions at the 3-alpha, 20-alpha, and 17-beta positions of steroids (1). It is also known as Prostaglandin F synthase as it reduces prostaglandin D2 to F2, and therefore may play a role in allergic conditions such as asthma (2). Elevated expression of AKR1C3 in endometrium that results in enhanced estrogen action may lead to endometrial cancer (3). It is also up-regulated in squamous cell carcinoma of head and neck (4). AKR1C3 is found to be a novel suppressor of cell differentiation that provides a plausible target for the non-cyclooxygenase-dependent antineoplastic actions of nonsteroidal anti-inflammatory drugs (5).

References:

1. Penning, T. M. *et a*l. (2000) Biochem J. **351**:67.
2. Suzuki-Yamamoto, T. *et al.* (1999) FEBS Lett. **462**:335.
3. Rizner, T. L. *et al.* (2006) Mol. Cell. Endocrinol. **248**:126.
4. Li, S. *et al.* (2004) Br. J. Cancer **90**:1093.
5. Desmond, J. C. *et al.* (2003) Cancer Res. **63**:505.

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