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11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2) Assay Kit (Cat #: E-152)

COMPONENTS: 11β-HSD2 Assay Solution- 5 ml (for 100 wells), store at -80°C **(shield solution from light during assay)**
10x 11β-HSD2 Control- 0.5 ml, store at 4°C
10x 11β-HSD2 Reaction- 0.5 ml, store at 4°C
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The 11β-HSD2 enzyme activity assay is based on the oxidation of cortisol (corticosterone), which is coupled to NADH-dependent reduction of INT to formazan. The formazan product exhibits an absorption maximum at 492 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$), allowing for measurement of 11β-HSD2 activity present in cell/tissue extracts and serum. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~5 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

Reagent thawing:

11β-HSD2 Assay Solution: Keep thawed assay solution on ice and shielded from light. *Do not over thaw*. Freeze assay solution immediately after use.

10x 11β-HSD2 Control and Reaction: Keep both solutions at room temperature prior to assay. Vortex solution to dissolve precipitates if necessary.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of 10x 11β-HSD2 Control and 10 parts of 11β-HSD2 Assay Solution, e.g. 50 µl 11β-HSD2 Control mixed with 500 µl 11β-HSD2 Assay Solution.

Reaction solution is prepared by mixing 1 part of 10x 11β-HSD2 Reaction and 10 parts of 11β-HSD2 Assay Solution, e.g. 50 µl 11β-HSD2 Reaction mixed with 500 µl 11β-HSD2 Assay Solution.

Enzyme assay:

1. Keep thawed samples on ice. *Do not over thaw.* Add 20 µl of each cell/tissue lysate to duplicate wells of a plain (uncoated) 96-well plate.
2. Add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C humidified incubator for 60 min. *Do not use CO_2 incubator.*
3. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
4. Sample 11β-HSD2 enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl / (60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1**. Enzyme activity may be normalized by GAPDH enzyme activity (#E-101) or protein concentration (#A-117).

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). 10x 11β-HSD2 Reaction contains cortisol. Please refer to the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Redissolve contents by agitation in warm water upon arrival.