BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Enolase Assay kit (Cat #: E-153)

COMPONENTS: Enolase Substrate: 0.15 ml, store at -80°C
ATP Assay Solution: 10 ml (100 assays), store at -80°C **(shield solution from light during use)**
PK Buffer: 0.5 ml, store at -80°C
PK Enzyme: 20 µl, store at 4°C **(close tube tightly to prevent dehydration; do not freeze)**
TCA Stop: 1 ml, store at 4°C **(caution: avoid contact with skin)**
10x Cell Lysis Solution: 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The Enolase Assay Kit is based on chemiluminescence detection of ATP. Enzyme activities present in cell/tissue lysates, cell culture medium, and serum can be assayed by measurement of Relative Light Unit (RLU) using a luminometer. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue and serum samples:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~10^5 freeze-thawed 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. Fresh or freeze-thawed tissue is homogenized in ice-cold 1x Cell Lysis Solution (~2 mg tissue in 0.5 ml).
2. Centrifuge lysate in a refrigerated microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and kept on ice. Use the BCA protein assay method to determine protein concentration. Adjust sample protein concentration to 0.05 - 0.5 mg/ml using ice-cold 1x Cell Lysis Solution as diluent. *Normalization of sample protein concentration is recommended.* Store samples at -80ºC.
3. Serum samples should be diluted 3-fold with ice-cold dH_2O for the assay. *Do not use plasma or undiluted serum for the assay.*

Preparation of PK mix and Reaction solution:

Gently vortex PK Enzyme vial before pipetting. Add 1 µl PK enzyme to 50 µl PK Buffer followed by mixing; this is PK mix. Prepare Reaction solution by mixing an equal volume of Enolase Substrate and PK mix. Keep solution on ice during assay.

Enzyme assay:

1. Keep thawed Enolase Substrate and ATP Assay Solution on ice. *Do not over thaw*. Shield ATP Assay Solution from light during assay.
2. Add 2.5 µl Reaction solution to each of a set of 0.5-ml microtubes and keep on ice.
3. For assay control, add 2.5 µl 1x Cell Lysis Solution and 5 µl TCA Stop to a designated tube containing 2.5 µl Reaction solution. Mix contents and place the control tube on ice.
4. Proceed to analyze the first sample by adding 2.5 µl lysate to 2.5 µl Reaction solution and mix by pipetting up and down twice. Immediately place tube in a 37°C-water bath for exactly 1 min (for cell/tissue lysate) or 5 min (for cell culture medium/diluted serum). Remove tube from water bath and immediately terminate reaction by adding 5 µl TCA Stop. Vortex tube and place tube on ice. *Reaction time may be optimized by end users.*
5. Repeat Step 4 for the next sample and so on. Keep reaction time the same for all samples. All sample tubes should be kept on ice for at least 10 min after addition of TCA Stop.
6. Centrifuge control and sample tubes in a microfuge for 5 min (~14,000 rpm).
7. Remove tubes from centrifuge. Add 0.25 ml ice-cold dH_2O to each tube. Mix solution and centrifuge for 3 min.
8. Transfer 10 µl of supernatant from each tube to a clear (uncoated) 96-well plate for measurement of Relative Light Unit (RLU) using a luminometer. Add 0.1 ml ATP Assay Solution to each well and record RLU.
9. Subtract control RLU from sample RLU to obtain **ΔRLU** for each sample. Enolase activity is represented by **ΔRLU**, which can be normalized by protein concentration or GAPDH enzyme activity of each sample. *Increase sample protein concentration or reaction time at Step 4 if low ΔRLU is obtained.*

Additional information:

- Please visit the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.