



TECHNICAL MANUAL

Enolase Activity Assay Kit

- **SKU CODE:** BR00006
- **SIZE:** 100 assays
- **DETECTION PRINCIPLE:** Chemiluminescent
- **RUO:** Research-Use-Only

Enolase Activity Assay Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

100 assays

Measuring instrument:

Luminometer (Relative Light Unit, RLU)

Sample type:

Cell/tissue lysates, cell culture medium, and serum

2. Storage & Expiry

This product should be stored according to the conditions listed for each kit component. Kit components are stable for at least one year if stored and handled properly. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Enolase Activity 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.

4. Kit Contents

No	Component Name	Size	Storage
1	Enolase Substrate	0.15 ml	-80°C
2	ATP Assay Solution	10 ml (100 assays)	-80°C (shield solution from light during use)
3	PK Buffer	0.5 ml	-80°C
4	PK Enzyme	20 µl	4°C (close tube tightly to prevent dehydration; do not freeze)
5	TCA Stop	1 ml	4°C (caution: avoid contact with skin)
6	10x Cell Lysis Solution	25 ml	Room temperature (agitate vial to redissolve contents)

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

1. 37°C water bath.
2. Luminometer (Relative Light Unit, RLU).
3. Clear (uncoated) 96-well plate.
4. 0.5-ml microtubes and disposable tubes for sample dilution.
5. Refrigerated microcentrifuge (~14,000 rpm) and vortex mixer.
6. Precision pipettes and disposable pipette tips. Distilled water.
7. BCA protein assay reagents.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We

advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. Please visit the product webpage or contact Assay Genie for material SDS information.
8. 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.

6. Reagent Preparation

1. **1x Cell Lysis Solution:** Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH₂O.
2. **PK mix:** Gently vortex PK Enzyme vial before pipetting. Add 1 µl PK Enzyme to 50 µl PK Buffer followed by mixing; this is PK mix.
3. **Reaction solution:** Prepare Reaction solution by mixing an equal volume of Enolase Substrate and PK mix. Keep solution on ice during assay.
4. **Enolase Substrate and ATP Assay Solution:** Keep thawed Enolase Substrate and ATP Assay Solution on ice. Do not over thaw. Shield ATP Assay Solution from light during assay.

7. Sample Preparation

A. Sample Preparation

Cell/tissue samples

1. Bring up ~10⁵ 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.

3. 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 -80°C.

Serum samples

1. Serum samples should be diluted 3-fold with ice-cold dH₂O for the assay. Do not use plasma or undiluted serum for the assay.

B. Dilution

Sample Type	Dilution factor
Serum	3-fold

Note: The recommended diluent is ice-cold dH₂O. For sample types not specified in the protocol, it is advised to perform a preliminary test to determine the appropriate dilution factor or contact our Tech Support Team at techsupport@assaygenie.com.

8. Assay Procedure

1. **Prepare reaction tubes:** Add 2.5 µl Reaction solution to each of a set of 0.5-ml microtubes and keep on ice.
2. **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.
3. **Sample reaction:** 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.
4. **Remaining samples:** Repeat Step 3 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.

5. **Centrifuge:** Centrifuge control and sample tubes in a microfuge for 5 min (~14,000 rpm).
6. **Dilute:** Remove tubes from centrifuge. Add 0.25 ml ice-cold dH₂O to each tube. Mix solution and centrifuge for 3 min.
7. **Measure:** 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. Data Analysis

Calculation:

$$\Delta\text{RLU} = \text{RLU (sample)} - \text{RLU (control)}$$

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 3 of the Assay Procedure if low ΔRLU is obtained.

Note:

RLU: *Relative Light Unit.*

ΔRLU : *sample RLU minus control RLU.*

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