LDH-A Enzyme Assay

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[Abstract] LDH (Lactate dehydrogenase) enzyme catalyzes the reversible conversion of pyruvate to lactate using NAD+ as a cofactor. Although the physiological significance of lactate accumulation in tumor cells, a dead-end product in cellular metabolism, is currently a topic of debate, it has long been known that many tumor cells express a high level of LDH-A (Koukourakis *et al*., 2003; Koukourakis *et al*., 2006; Koukourakis *et al*., 2009). So detection of its enzyme activity *in vitro* is important for researching on LDH-A. Recently, it has been reported that Lys-5 acetylation could decrease LDH-A enzyme activity (Zhao *et al*., 2013).

Materials and Reagents

1. 293T cells
2. DMEM + 10% NCS
3. Aprotinin (BBI Solutions, catalog number: AD0153-50mg)
4. Leupeptin (AMRESCO, catalog number: J580-25MG)
5. Pepstatin (AMRESCO, catalog number: J583)
6. PMSF (Sangon Biotech, catalog number: P0754-5g)
7. Tris-HCl (pH 7.3) (Sangon Biotech)
8. 250 μg/ml Flag peptide (in PBS buffer) (GL Biochem, sequence: DYKDDDDK)
9. Pyruvate (Sigma-Aldrich, catalog number: 80443)
10. NADH (Sigma-Aldrich, catalog number: N8129)
11. Flag-beads (Sigma-Aldrich, catalog number: M8823)
12. Lipofectamine 2000 (Invitrogen)
13. Reaction buffer (see Recipes)
14. 0.3% NP-40 buffer (Lysis buffer) (see Recipes)

Equipment

1. F-4600 Fluorescence Spectrophotometer
2. 37 °C, 5% CO_2 incubator
3. 90 mm cell culture plate

Procedure

1. Prepare LDH-A protein. You could ectopically overexpress and purify it from *E. coli*, or ectopically express Flag-LDH-A plasmid in 293T cells, followed by immunoprecipitation by Flag-beads and eluted using Flag peptide.
2. 293T cells were cultured in DMEM + 10% NCS, in 5% CO_2 incubator at 37 °C. Cell transfection was performed using Lipofectamine 2000 or calcium phosphate methods. 2 μg plasmids was transfected into 90 mm plate of 293T cells. And cells were cultured for 30 hours after transfection.
3. Cells ectopically expressed Flag-LDH-A were lysated by 0.3% NP-40 buffer (lysis buffer) by shaking gentally at 4 °C for half an hour.
4. Cell lysate was centrifuged 4 °C for 15 min (16,000 ×g) and the supernatant was incubated with 10 μl (per 90 mm plate of 293T cells) Flag-beads for 3 hours at 4 °C by rotation slowly.
5. And then flag-beads were washed and centrifuged at 4 °C for 1 min (400 *x g*) by 1 ml 0.3% NP-40 buffer for 3 times, and incubated with 250 μg/ml of flag peptide (200 μl per 90 mm plate of 293T cells) shaking for one hour, followed by centrifuge at 16,000 ×g for 5 min. The supernatant was used for enzyme activity detection.
6. Prepare the reaction buffer containing 0.2 M Tris-HCl (pH 7.3), 30 mM pyruvate and 6.6 mM NADH.
7. For every reaction, 10 μl LDH-A enzyme solution and 290 μl reaction buffer are added into the measuring cup of F-4600 Fluorescence Spectrophotometer, and detect the fluorescence change in absorbance (340 nm) resulting from NADH oxidation at room temperature.

   Note: The reaction system could be adjusted according to your LDH-A solution concentration. And the reaction is very quick, please detect the change as soon as possible.
8. After a reaction, the software will show the slope of fluorescence change, and this value is the speed of this reaction.

Recipes

1. Reaction buffer
   0.2 M Tris-HCl (pH 7.3)
   30 mM pyruvate
   6.6 mM NADH
2. 0.3% NP-40 buffer (Lysis buffer)
   50 mM Tris-HCl (pH 7.5)
   150 mM NaCl
   0.3% Nonidet P-40
   1 μg/ml aprotinin
   1 μg/ml leupeptin
   1 μg/ml pepstatin
   1 mM PMSF

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