LDH-A Enzyme Assay

Di Zhao[1], Yue Xiong[2], Qun-Ying Lei[3] and Kun-Liang Guan[4*]

[1]Department of Biochemistry and Molecular Biology, Fudan University Shanghai Medical College, Shanghai, China; [2]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, USA; [3]Department of Biochemistry and Molecular Biology, Fudan University, Shanghai, China; [4]Department of Pharmacology and Moores Cancer Center, University of California, San Diego, USA

*For correspondence: kuguan@ucsd.edu

[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

Acknowledgments

This protocol has been adapted from the previously published paper Zhao *et al.* (2013) and is described in further detail. We thank the members of the Fudan MCB laboratory for discussions throughout this study. We also thank Dr. Liming Wei for the IEF assay. This work was supported by the Chinese Ministry of Sciences and Technology 973 (Grant No. 2009CB918401, 2011CB910600), (Grant No. NCET-09-0315), NSFC (Grant No.31271454, 81225016) and NSFC-NIH (Grant No. 81110313). This work was also supported by Chinese Ministry of Education 985 Program, 100 Talents Program of Shanghai Health and Scholar of "Dawn" Program of Shanghai Education Commission and Shanghai Key basic research program(12JC1401100) to Q.Y.L. and NIH grants (Y.X. and K.L.G.); and the Fudan University Medical School Graduate Student Ming Dao Project Funds (D.Z.). This work is dedicated to the memory of Zhen Yu, who prepared the K5 acetylation antibody.

References

1. Koukourakis, M. I., Giatromanolaki, A., Sivridis, E., Bougioukas, G., Didilis, V., Gatter, K. C., Harris, A. L., Tumour and Angiogenesis Research, G. (2003). Lactate dehydrogenase-5 (LDH-5) overexpression in non-small-cell lung cancer tissues is linked to tumour hypoxia, angiogenic factor production and poor prognosis. *Br J Cancer* 89(5): 877-885.
2. Koukourakis, M. I., Giatromanolaki, A., Sivridis, E., Gatter, K. C., Harris, A. L. and Tumour

Angiogenesis Research, G. (2006). Lactate dehydrogenase 5 expression in operable colorectal cancer: strong association with survival and activated vascular endothelial growth factor pathway--a report of the Tumour Angiogenesis Research Group. *J Clin Oncol* 24(26): 4301-4308.

3. Koukourakis, M. I., Kontomanolis, E., Giatromanolaki, A., Sivridis, E. and Liberis, V. (2009). Serum and tissue LDH levels in patients with breast/gynaecological cancer and benign diseases. *Gynecol Obstet Invest* 67(3): 162-168.
4. Zhao, D., Zou, S. W., Liu, Y., Zhou, X., Mo, Y., Wang, P., Xu, Y. H., Dong, B., Xiong, Y., Lei, Q. Y. and Guan, K. L. (2013). Lysine-5 acetylation negatively regulates lactate dehydrogenase A and is decreased in pancreatic cancer. *Cancer Cell* 23(4): 464-476.

