Cell Biosciences™

NanoPro™ Assay: Lactate Dehydrogenase (LDH)

APPLICATION BRIEF No. 1028

SUMMARY

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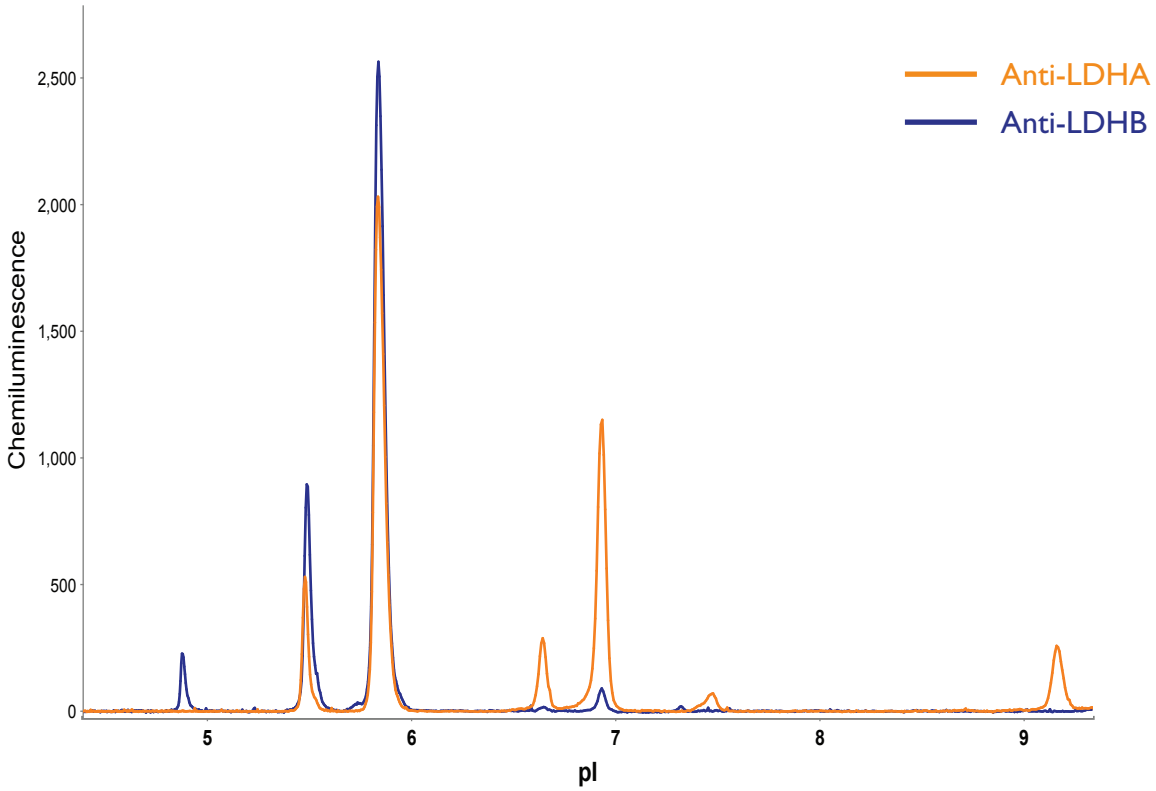
Primary Antibody: Anti-LDHA (Cell Signaling Technology, cat# 3582) and Anti-LDHB (Abcam, cat# ab85319)
Detection Antibody: Anti-Mouse HRP and Anti-Rabbit HRP (Cell Biosciences, p/n 040-655 and p/n 040-657)

Lactate dehydrogenase (LDH) is a reversible enzyme that catalyzes the reduction of pyruvate to lactate or the oxidation of lactate to pyruvate. In most human cells, LDH in its native form is a tetramer with 5 possible isoforms (LDH1-5), combinations of LDHA (calculated pI: 8.4) and/or LDHB (calculated pI: 5.7). LDH overexpression and differential expression of LDH isoforms have been implicated in the pathogenesis and progression of many cancers.

RESULTS



LDHA and LDHB in WM35 cells

The NanoPro technology using native conditions allows investigation of complexes, as shown by the examples of LDHA and LDHB.

The peaks at pI 9.2 and 4.9 were recognized only by the LDHA (Cell Signaling Technology, cat# 3582) or LDHB (Abcam, cat# ab85319) antibody and consequently were identified as LDH5 (LDHAx4) and LDH1 (LDHBx4), respectively. Peak identification was also supported by LDH standards and denaturing experiments (data not shown). The peaks with intermediate pI values were recognized by both LDHA and LDHB antibodies, indicating that these complexes were composed of both LDHA and LDHB proteins.

NOTE: Detection of the chemiluminescent signal produced is relative. Absolute units may vary depending on cell line, treatment and assay conditions.

PROTOCOL

Cell Preparation

Cell culture: WM35 cells (MD Anderson Cancer Center Core Facility) were cultured in RPMI 1640 (Mediatech, Inc, cat# 10-040-CV) containing 5% FBS (Gibco, cat# 10437). Cells were split 1:10 every 5 days using 1x Trypsin EDTA (Mediatech, Inc, cat#25-053-CI) at 37 °C for 3-5 minutes. Data shown from cells at passage 7.

Lysis buffer: Bicine/CHAPS Lysis Buffer (Cell Biosciences, p/n 040-764) plus 2 mM EDTA.

Lysis details: Wash cells with 10 mL of ice-cold PBS (HyClone, cat# SH30256.01), aspirate well. Add 400 µL ice-cold lysis buffer to 10-cm plate on ice, swirl around to ensure good coverage, and incubate 10 minutes on ice. Scrape plate, pipet up and down to mix. Transfer lysate to microfuge tube, lyse for an additional 20 minutes on ice. Clarify by centrifugation (14,000 x g, 15 minutes) in a cooled centrifuge. Transfer supernatant to a fresh microfuge tube. Immediately aliquot supernatant (10-30 µL) on ice and snap freeze on dry ice.

Storage: -80 °C

Assay Reagents

NOTE: For specifics on sample preparation, please consult the addendum to this document.

Protein concentration: 0.02 mg/mL final in capillary by BCA assay

Sample diluent: Bicine/CHAPS Lysis Buffer plus 1x DMSO Inhibitor Mix

Ampholyte premix: Premix 3-10 (Cell Biosciences Premix G1, p/n 040-806 or Premix G2, p/n 040-968)

pI standards: pI Standard Ladder 1 (Cell Biosciences, p/n 040-644), spike in pI standard 9.7 (Cell Biosciences, p/n 040-790)

Wash: Wash Buffer (Cell Biosciences, p/n 040-654)

Primary antibody: Anti-LDHA (Cell Signaling Technology, cat# 3582), 1:50 and Anti-LDHB (Abcam, cat# 85319), 1:500 in Antibody Diluent (Cell Biosciences, p/n 040-309)

Detection antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656) and Anti-Mouse HRP (Cell Biosciences, p/n 040-655) in Antibody Diluent

Anolyte: Phosphoric Acid, 10 mM (Cell Biosciences, p/n 040-650)

Catholyte: Sodium Hydroxide, 100 mM (Cell Biosciences, p/n 040-651)

Luminol/Peroxide: Mixed 1:1 (Cell Biosciences, p/n 040-652 and p/n 040-653)

NOTE: NanoPro XDR Assays are now available with optional reagents to provide optimized assay sensitivity, extended dynamic range and both low and high end signal enrichment. Additionally, Premix G2 provides enhanced protein resolution. For more information, visit cellbiosciences.com, contact your Cell Biosciences' Field Applications Specialist or call Technical Support at (888) 607-9692.

Assay Conditions

System:	NanoPro 1000
Sample loading time:	10 seconds (Premix G1), 25 seconds (Premix G2)
Focus conditions:	15000 µW, 40 minutes (Premix G1) or 21000 µW, 40 minutes (Premix G2)
Immobilization:	Anti-LDHA: 240 minutes, Anti-LDHB: 60 minutes
Wash 1:	2 x 150 seconds (default)
Primary antibody incubation:	60 minutes
Wash 2:	2 x 150 seconds (default)
Detection antibody incubation:	60 minutes
Wash 3:	2 x 150 seconds (default)
Chemiluminescence exposure:	60, 120, and 240 seconds

Our favorite antibodies

Anti-LDHA (Cell Signaling Technology, cat# 3582)
Anti-LDHB (Abcam, cat# ab85319)

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SAMPLE PREPARATION ADDENDUM

Sample preparation procedures are different depending on the premix used. The following updated instructions provide guidance on sample preparation for both Premix G1 and Premix G2.

Step	Premix G1 Procedure	Premix G2 Procedure
Step 1	Dilute lysate with sample diluents to 0.1 mg/mL.	Dilute lysate with sample diluents to 0.2 mg/mL.
Step 2	In a separate tube, mix Premix G1and pI standards.	In a separate tube, mix Premix G2 and pI standards.
Step 3	Mix equal parts of diluted lysate prepared in Step 1 with the Premix G1 + pI Standards prepared in Step 2 (1:1 ratio) to create final protein concentration of 0.05 mg/mL.	Mix 1 part diluted lysate prepared in Step 1 with 3 parts Premix G2 + pI Standards prepared in Step 2 (1:3 ratio) to create final protein concentration of 0.05 mg/mL.

NOTES: When working with Premix G2, thorough mixing and vortexing during sample preparation is required.
Additional sample volume may be required, 12-20 uL per sample well is recommended.
Centrifugation of the sample plate (3000 x g, 10 minutes) is required.

For further assistance, please contact your Cell Biosciences' Field Applications Specialist or Technical Support at (888) 607-9692.

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