Cell Biosciences™

NanoPro™ Assay: Asparagine Synthetase (ASNS)

APPLICATION BRIEF No. 1027

SUMMARY

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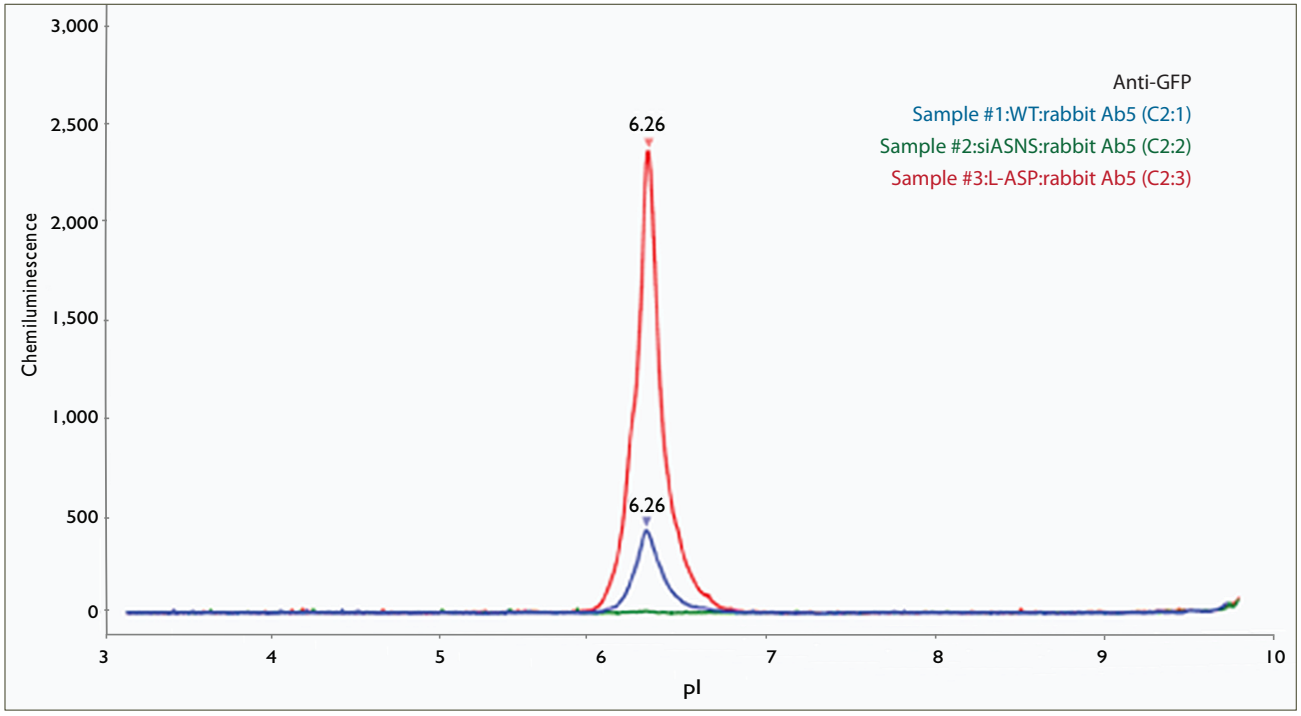
Primary Antibody: Anti-ASNS (Sigma, cat# HPA029318)
Detection Antibody: Anti-Mouse HRP and Anti-Rabbit HRP (Cell Biosciences, p/n 040-655 and p/n 040-657)

The enzyme-drug L-asparaginase has been used since the 1970s to treat acute lymphoblastic leukemia. Asparagine synthetase (ASNS) expression has been found to be correlated with L-asparaginase efficacy in leukemia cell lines, in leukemia primary tumor samples, and more recently in cancer cell lines from other tissues of origin. Silencing ASNS expression by RNAi has indicated the L-asparaginase/ASNS relationship is causal and suggests that ASNS expression may be useful as a predictive clinical biomarker of L-asparaginase efficacy. ASNS presents as a single peak in the NanoPro assay. Expected changes of expression are observed upon siRNA as well as L-asparaginase treatment.

RESULTS



Asparagine synthetase (ASNS) in OVCAR-8 cells

ASNS in OVCAR-8 cells (US National Cancer Institute) presents in the NanoPro assay as a single peak around pI 6.3 using the protocol described. ASNS expression was stimulated by cell treatment with L-asparaginase as well as inhibited by specific siRNA treatment.

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: OVCAR-8 cells (US National Cancer Institute) were cultured in RPMI-1640 (Thermo/Hyclone, cat# SH30096FS) containing 5% FBS (Thermo/Hyclone, cat#SH30070.01) and 2 mM Glutamine (Thermo/Hyclone, cat# SH3003401). Cells were split 1:10 every 3 days using 0.05% Trypsin (Thermo/Hyclone #SH3023601) at 37 °C for 3–5 minutes. Data shown from cells at passage 7.

Pre-treatment: Cells were seeded with either 5 nM ASNS siRNA complexed with INTERFERin transfection reagent (PolyPlus Transfection, VWR, cat# 89129-930) or corresponding negative control siRNA (QIAGEN, cat#1027281).

Treatment: Cells were fed with fresh medium containing 0.1 U/mL of L-asparaginase (Sigma, cat# A3809) or vehicle control at 48 hours after seeding for a duration of 48 hours.

Lysis buffer: Bicine/CHAPS Lysis Buffer (Cell Biosciences, p/n 040-764) plus 1x DMSO Inhibitor Mix (Cell Biosciences, p/n 040-510) and 1x Aqueous Inhibitor Mix (Cell Biosciences, p/n 040-482).

Lysis details: Wash cells with 10 mL of ice-cold Wash Buffer (Cell Biosciences, p/n# 040-313), 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 (20,000 x g, 10 minutes) in a cooled centrifuge. Transfer supernatant to a fresh microfuge tube. Immediately aliquot supernatant (10–30 µL) on ice and snap freeze on liquid nitrogen.

Storage: -80 °C

Assay Reagents

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

Protein concentration:	0.05 mg/mL final in capillary by BCA assay
Sample diluent:	Bicine/CHAPS Lysis Buffer plus 1x DMSO Inhibitor Mix
Ampholyte premix:	Premix 5-7 (PGM) (Serva, cat# 42936.01) at 12% in ampholyte-free Premix G2 (Cell Biosciences, p/n 040-967)
pI standards:	Custom ladder containing pI Standards 4.9, 5.5, 6.0, 6.4, and 7.0 (Cell Biosciences, p/n 040-027, p/n 040-028, p/n 040-031), 1:100
Wash:	Wash Buffer (Cell Biosciences, p/n 040-654)
Primary antibody:	Anti-ASNS (Sigma cat# HPA029318) at final 5 µg/mL in Antibody Diluent (Cell Biosciences, p/n 040-309)
Detection antibody:	Anti-Rabbit HRP (Cell Biosciences, p/n 040-656), 1:100 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:	25 seconds
Focus conditions:	21000 µW, 40 minutes
Immobilization:	40 seconds
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 antibody

Anti-ASNS (Sigma, cat# HPA029318)

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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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