NanoPro™ Assay: Green Fluorescent Protein (GFP)

SUMMARY

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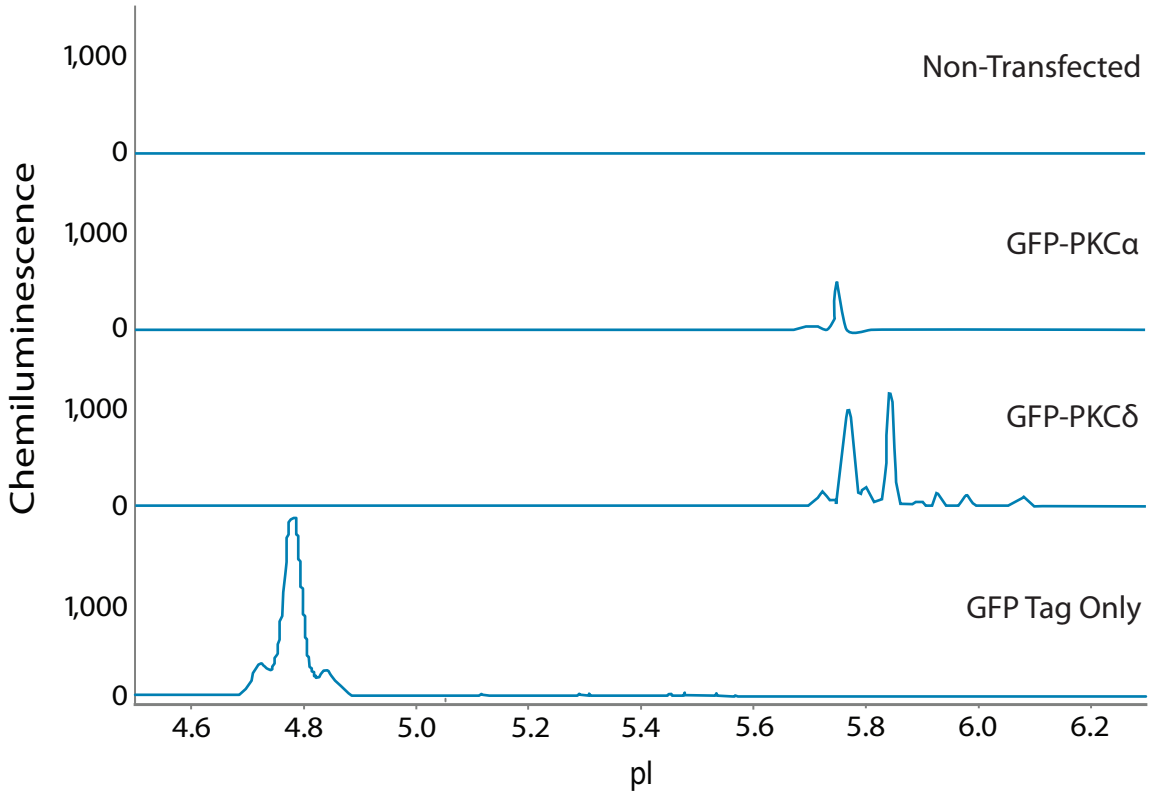
Primary Antibody: Anti-GFP (Roche Applied Science, cat# 11814460001)
Detection Antibody: Anti-Mouse HRP (ProteinSimple, p/n 040-655)

Green Fluorescent Protein (GFP) was originally isolated from jellyfish and exhibits a bright green fluorescence when exposed to blue light. In cell and molecular biology, GFP is commonly utilized as a reporter of protein expression. GFP can be introduced and its expression maintained in a wide range of cell lines and organisms. Thus, assays suitable for GFP detection offer useful tools for following the expression of proteins for which high affinity aniibodies are lacking. The data demonstrates identification of a highly sensitive antibody for GFP detection via NanoPro assay.

RESULTS



DETECTION OF GFP IN TRANSFECTED LNCAP CELLS

LNCap cells were transfected with 4 µg plasmid encoding GFP-PKCα, GFP-PKCδ, or GFP only. The anti-GFP antibody detects GFP peaks corresponding to expression of protein encoded by the plasmid utilized for transfection. GFP-PKC peaks also detected at identical pI by an anti-PKC-specific antibody (data not shown).

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

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PROTOCOL

CELL PREPARATION	
Cell culture:	LNCaP human prostate cells (ATCC, cat# CRL-1740) were cultured in RPMI-1640 (ATCC, cat# 30-2001) and supplemented with 10% FBS (ATCC, cat# 30-2020) and 2 mM glutamine (ATCC, cat# 30-2214) in a humidified incubator in 5% CO_2. Cells were split 1:4 every 3 days using 0.25% Trypsin (Invitrogen, cat# 25-200) at 37 ˚C for 3 – 5 minutes.
Transfection:	Two days after plating into 6 cm plates, cells were transfected with 4 µg of plasmid using Lipofectamine and Plus reagent (Invitrogen, cat# 18324-012 and cat# 11514-015 respectively) following the manufacturer's instructions. The transfected plasmids were pEGFN1 for GFP (Clontech, discontinued), GFP-PKCα (bovine) and GFP-PKCδ (mouse) (Kedei et al., 2004*). 4 µg DNA was mixed with 8 µL Plus reagent in 250 µL OPTI-MEM (Invitrogen, cat# 31985), 12 µL Lipofectamine was diluted in 250 µL OPTI-MEM, combined after 15 minutes, and added to the cells containing 2 mL of OPTI-MEM (3 mL cell culture medium was replaced with 2 mL OPTI-MEM). After 3 – 4 hrs incubation, the transfection mixture was replaced with normal cell culture medium.
Treatment:	48 hours after transfection, cell culture medium was removed, cells were washed once in 3 mL PBS (Cellgro, cat# 21-031-CV) and collected in 1 mL PBS using a cell scraper (Sarstedt, cat# 83.1830). Cells were centrifuged at 4000 RPM for 5 minutes using a cooled-table Eppendorf centrifuge (5417R). Supernatant was removed using vacuum, and the pellet was kept at – 80 °C until further use.
Lysis buffer:	RIPA Lysis Buffer (ProteinSimple, p/n 040-483) plus 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510) and 1x Aqueous Inhibitor Mix (ProteinSimple, p/n 040-482).
Lysis details:	200 µL ice-cold lysis buffer (containing protease and phophatase inhibitors) was added to 3 x 10^6 cells, resuspended and incubated 20 minutes by rotating in a cold room. Lysates were clarified in a chilled centrifuge (14,000 x g, 15–20 minutes). Supernatants were aliquoted for storage.
Storage:	–80 °C

* Kedei N, Lundberg DJ, Toth A, Welburn P, Garfield SH, Blumberg PM. Characterization of the interaction of ingenol 3-angelate with protein kinase C. Cancer Res. 2004 May 1;64(9):3243-55.

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ASSAY REAGENTS

Protein concentration:	0.01 – 0.1 mg/mL final in capillary by Pierce 660 nM Protein Assay
Sample Diluent:	M-Per Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, cat# 78501) plus 4x DMSO Inhibitor Mix (comparable to Bicine/CHAPS Lysis Buffer, ProteinSimple, p/n 040-764)
Ampholyte Premix:	G2 Premix 5 – 8 (nested) (ProteinSimple, p/n 040-972)
pI Standards:	pI Standard Ladder 3 (ProteinSimple, p/n 040-646), pI Standard 5.5 (ProteinSimple p/n 040-028)
Wash:	Wash Buffer (ProteinSimple, p/n 040-654)
Primary antibody:	Anti-GFP (Roche Applied Science, cat# 11814460001), 1:100 in Antibody Diluent (ProteinSimple, p/n 040-309)
Detection antibody:	Anti-Mouse HRP (ProteinSimple, p/n 040-655) 1:100 in Antibody Diluent
Anolyte:	Phosphoric Acid, 10 mM (ProteinSimple, p/n 040-650)
Catholyte:	Sodium Hydroxide, 100 mM (ProteinSimple, p/n 040-651)
Luminol/Peroxide xDR:	Mixed 1:1 (ProteinSimple, p/n 040-652 and p/n 041-084)

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 proteinsimple.com, contact your ProteinSimple Field Applications Specialist or call Technical Support at (888) 607-9692.

SAMPLE PREPARATION

Step 1	Dilute lysate with sample diluents to 0.2 mg/mL.
Step 2	In a separate tube, mix Premix G2 and pI standards.
Step 3	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

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ASSAY CONDITIONS

System:	NanoPro 1000
Sample Loading Time:	25 seconds
Focus Conditions:	21000 µW, 40 minutes
Immobilization:	80 seconds
Wash 1:	2 x 150 seconds (default)
Primary antibody incubation:	90 minutes
Wash 2:	2 x 150 seconds (default)
Detection antibody incubation:	60 minutes
Wash 3:	2 x 150 seconds (default)
Chemiluminescence exposure:	60, 120, 240 and 480 seconds

OUR FAVORITE ANTIBODIES

Anti-GFP (Roche Applied Science, cat# 11814460001)

OTHER ANTIBODY SUGGESTIONS

Anti-GFP (Invitrogen, cat# A11122)

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