Cell Biosciences™

NanoPro™ Assay: FLAG Epitope Tag on EGFP

APPLICATION BRIEF No. 1025

SUMMARY

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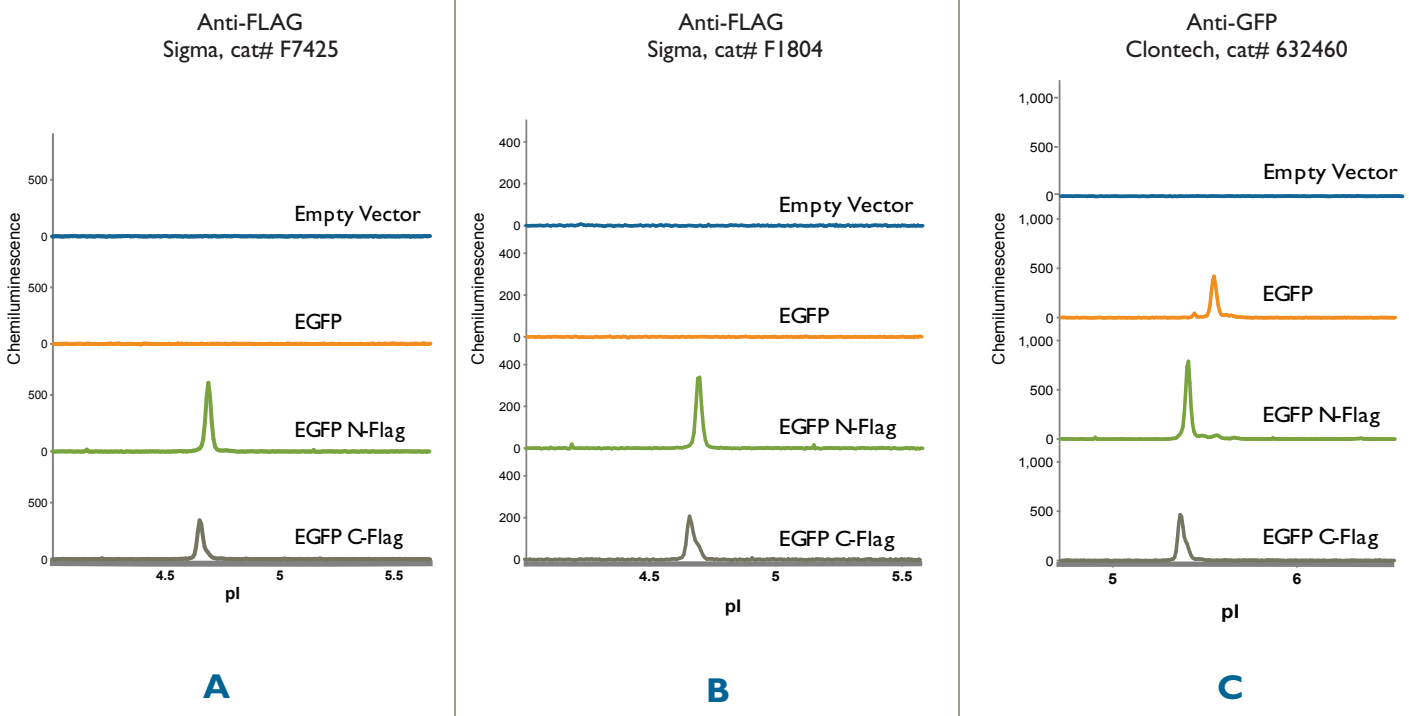
Primary Antibody: Anti-FLAG (Sigma, cat# F1804 and cat# F7425) and Anti-GFP (Clontech, cat# 632460)
Detection Antibody: Anti-Mouse HRP and Anti-Rabbit HRP (Cell Biosciences, p/n 040-655 and p/n 040-657)

Epitope tags are widely used for afinity purification as well as for highly sensitive detection of recombinant proteins. The FLAG-tag consists of a short peptide sequence (MADYKDDDDKM). EGFP was expressed with a FLAG-tag at either the C- or N-terminus of the protein. As expected from the amino acid composition of this tag, a slight acidic shift in the pI of the tagged protein can be observed.

RESULTS



FLAG-tagged EGFP expressed in mouse L-cells

FLAG-tagged EGFP expressed in mouse L-cells was detected specifically by two anti-FLAG antibodies (panels A, Sigma# F7425; and B, Sigma# F1804). Specificity was confirmed by vector-only and EGFP-only transfections, which did not result in any signal with the anti-FLAG antibodies. As expected, the anti-GFP antibody (panel C, Clontech# 632460) resulted in a significant peak in the GFP-only transfection. The anti-GFP antibody recognized also as expected both FLAG-tagged EGFP constructs.

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: LTK, also known as L-cells (ATCC, cat# CRL-2648), a mouse fibroblast cell line similar to 3T3 cells, were cultured in DMEM containing 10% FBS and Penicillin/Streptomycin. Cells were split 1:100 every week using trypsin.

Constructs: Expression from EGFP vector backbone (Clontech, cat# 6084-1 and 6085-1) was driven by a CMV promoter. PCR reaction was run from this backbone to generate plasmids expressing EGFP with either an N- or C-terminal tag. All clones were verified by sequencing.

Tag sequence: FLAG: MADYKDDDDKM

Transfection: Cells were cultured in 6-well plates and transfected with Lipofectamine 2000 (Invitrogen, cat# 11668019), according to manufacturer's instructions. The applied constructs were Empty vector, EGFP or EGFP with an N- or C-terminal FLAG tag. The transfection efficiency was between 20% and 30%, judged by EGFP fluorescence.

Lysis buffer: Tissue Reagent I (Invitrogen, cat# FNN0071) with the addition of protease inhibitors (Roche, cat# 1 836153).

Lysis details: Wash cells once with ice-cold PBS, aspirate well. Add 100 µL of ice-cold lysis buffer per well. Swirl around to ensure good coverage. Incubate for 10 minutes on ice. Scrape off cells, transfer to a microfuge tube and clarify by centrifugation (17,000 x g, 30 minutes) in a cooled centrifuge, transfer supernatant to a fresh microfuge tube.

Storage: -80 °C

Assay Reagents

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

Protein concentration: 0.1 mg/mL final in capillary by BCA assay

Sample diluent: Sample Diluent (Cell Biosciences, p/n 040-649) plus 1x DMSO Inhibitor Mix

Ampholyte premix: Premix 4-9 (Cell Biosciences Premix G1, p/n 040-319 or Premix G2, p/n 040-969)

pI standards: pI Standard Ladder 1 (Cell Biosciences, p/n 040-644)

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

Primary antibody: Anti-FLAG (Sigma, cat# F7425 and F1804) and anti-GFP (Clontech, cat# 632460), both 1:100 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), both 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: 10 seconds (Premix G1), 25 seconds (Premix G2)

Focus conditions: 15000 µW, 40 minutes (Premix G1) or 21000 µW, 40 minutes (Premix G2)

Immobilization: 80 seconds

Wash 1: 2 x 150 seconds (default)

Primary antibody incubation: 120 minutes

Wash 2: 2 x 150 seconds (default)

Detection antibody incubation: 60 minutes

Wash 3: 2 x 150 seconds (default)

Chemiluminescence exposure: 10, 60, 120, 240, 600 and 1200 seconds

Our favorite antibodies

Anti-FLAG (Sigma, cat# F1804)
Anti-FLAG (Sigma, cat# F7425)

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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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