Validation Report #029762

Validation Date: 07/07/14

Summary

Antigen	Green Fluorescent Protein (GFP)
Catalog number	ABIN398304
Supplier	Chromotek
Supplier catalog number	3H9
Lot number	100316
Method validated	Western Blot
Laboratory	Moores Cancer Center, UC San Diego
Validation number	29762
Positive Control	293FT cells transduced with eGFP virus
Negative Control	untransduced 293FT cells
Notes	A major band was observed at the correct molecular weight in the two positive (eGFP-transduced) cell lysates. No major bands were observed in the negative control (untransduced cells). Some additional faint bands of lower molecular weight were also observed in the positive controls, this may be because the antibody was used at 1:500 instead of the recommended 1:1000 dilution. Microscope images of transduced cells are provided to demonstrate successful eGFP-transduction of cells.

Independent Results

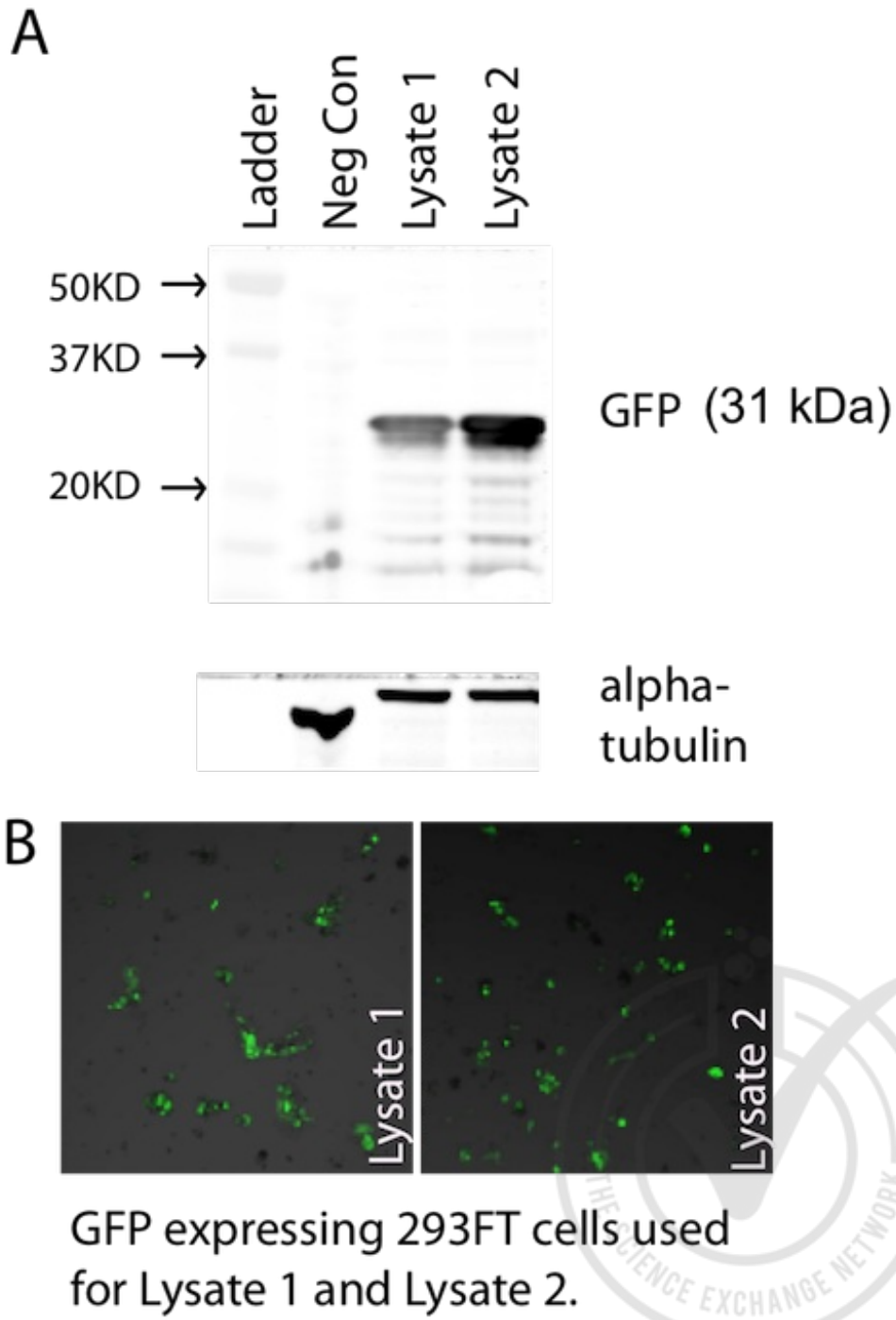

Figure 1: A. Western blot of lysates from untransduced 293FT cells (Lane 2) and 293FT cells transduced with eGFP virus (Lane 3 and 4) probed with anti-GFP antibody (upper panel) or for alpha tubulin (lower panel). B. Confocal image of 293FT cells used to generate Lysate1 and Lysate2.

Full Methods

Primary Antibody
- Antigen: Green Fluorescent Protein (GFP)
- Catalog number: ABIN398304
- Supplier: Chromotek
- Supplier catalog number: 3H9
- Lot number: 100316

Loading Control Antibody
- Antigen: alpha-tubulin (mouse monoclonal)
- Supplier: Sigma Aldrich
- Catalog number: T9026
- Lot number: N/A

Secondary Antibody
- Antibody: Alexa Fluor 680 Goat Anti-Rat IgG (H+L)
- Supplier: Life Technologies
- Catalog number: A-21096
- Lot number: N/A

Controls
- Positive control: 293FT cells transduced with eGFP virus
- Negative control: untransduced 293FT cells

Protocol
- 20 µg of total protein from prepared cell lysates was run on a 4-12% SDS-PAGE gel.
- The gel was run at 180 V for 40 minutes and transferred to a PVDF membrane using a wet-transfer apparatus.
- The membrane was rinsed in TBST for 10 min in TBST.
- The membrane was blocked in 3% BSA for 30 min.
- The membrane was incubated in 1:500 primary antibody diluted in 3% BSA at 4°C overnight.
- The membrane was washed 3 times for 10 min in TBST.
- The membrane was incubated in secondary antibody at 1:10000 for 1 hr at RT.
- The membrane was washed 3 times for 10 min in TBST.
- The membrane was imaged using a fluorescent imaging system at 679nm/702nm.

Experimental Notes

Fluorescent microscopy images are included to confirm eGFP transduction of 293FT cells.