Validation Report #028889

Validation Date: 11/07/13

Summary

Antigen	DYKDDDDK Tag
Catalog number	ABIN1112984
Supplier	Biospes
Supplier catalog number	BTL1005
Lot number	L21/2013G
Method validated	Immunofluorescence
Laboratory	Reveal Biosciences
Validation number	28889
Positive Control	HEK293 cells transfected with DYKDDDDK Tag
Negative Control	Untransfected HEK293 cells
Notes	Signal was detected in positive control samples but not in negative control samples.

Full Methods

Primary Antibody
- Antibody: DYKDDDDK Tag antibody
- Catalog number: ABIN1112984
- Supplier: Biospes
- Supplier catalog number: BTL1005
- Lot number: L21/2013G

Secondary Antibody
- Antibody: Goat anti-mouse IgG (H+L) IF488 conjugated antibody
- Supplier: Biospes
- Supplier catalog number: BSA1041
- Lot number: S6/2013S

Controls
- Positive control: HEK293 cells from ATCC transfected with a DYKDDDDK tagged protein (specimen known to contain the target protein).
- Negative control: Untransfected HEK293 cells from ATCC (specimen known to not contain the target protein).
- Isotype control: HEK293 cells from ATCC transfected with a DYKDDDDK tagged protein treated with isotype mouse IgG control instead of the primary antibody. Any staining observed is due to non-specific binding of secondary antibody.
- Secondary antibody only control: HEK293 cells from ATCC transfected with a DYKDDDDK tagged protein treated with Goat anti-Mouse IF488 secondary antibody only. Any staining observed is due to non-specific binding of secondary antibody.

Protocol
- HEK293 cells were grown directly on coverslips and transfected with a DYKDDDDK tagged protein.
- Cells were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature (RT).
- Fixed cells were rinsed three times in PBS for 5 min each at RT.
- Cells were blocked in 1 X PBS / 5% normal goat serum / 0.3% Triton X-100 for 20 min at RT.
- Cells were incubated with primary antibody diluted 1:100 in Universal Antibody Dilution Buffer (Electron Microscopy Sciences, 25885-05) overnight at 4°C.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Cells were incubated with secondary antibody diluted 1:150 in Universal Antibody Dilution Buffer (Electron Microscopy Sciences, 25885-05) for 60 min at RT in dark.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Coverslips were mounted on slides with Fluoro-Gel II with DAPI (Electron Microscopy Sciences Cat. No: 17985-50).
- Stained cells were imaged with an EIDAQ 100 fluorescent microscope.

Experimental Notes

None

Figures

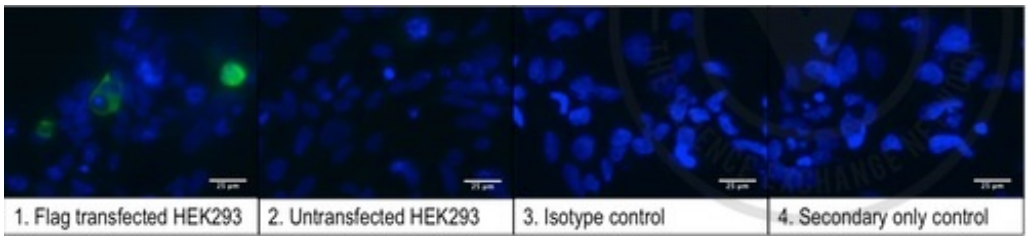

Figure 1: : Immunofluorescence analysis of cultured cells using DYKDDDDK Tag antibody (Catalog number ABIN1112984, Lot number L21/2013G). Flag is present in the positive control (HEK293 cells transfected with DYKDDDDK Tag panel 1, transfected cells are indicated by green fluorescent staining) and absent from the negative control (untransfected HEK293 cells panel 2). No staining was observed in the isotype (panel 3) and secondary only (panel 4) controls. DAPI was used as a nuclear counterstain (indicated by blue fluorescent staining). Scale bar = 25 µm.