MRC PPU
Reagents
and Services

ANTIBODY TESTING RESULTS
Standard Reporting Template

Antibody Name: Anti-IAV-PB1-F2

Full Antigen Name: PB1-F2 (accessory protein) protein of influenza A virus (IAV) strain A/Puerto Rico/8/1934(H1N1) ('PR8')

Antigen Species: Virus

Antigen: GST-IAV-PB1-F2 (1 – 87) DU73890, recombinantly expressed in Bacteria

Purification: serum heated treated, filtered and affinity purified against MBP-IAV-PB1-F2 (1 – 87) DU73898

Sheep Number: DA223

Bleeds Tested: 1st, 2nd, 3rd, 4th, 5th

Recommended Bleed: see results.

Immunoblotting:

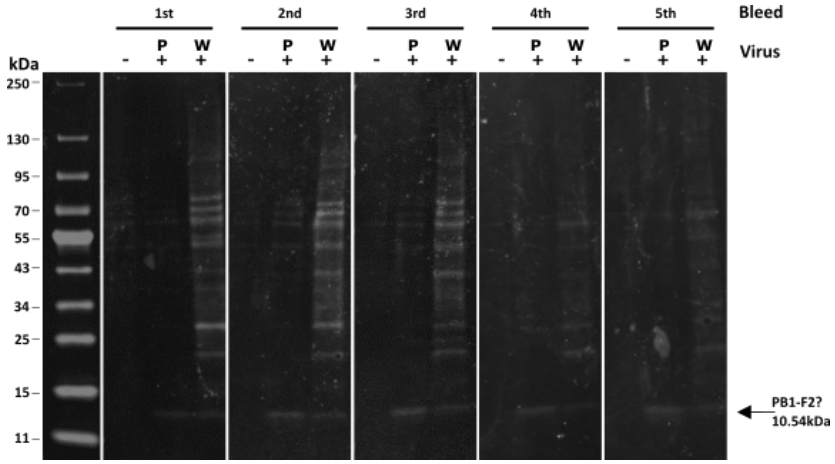
Method

MDCK cells were infected with IAV strains PR8 or A/WSN/33 ('WSN') at an MOI of 3 PFU/cell, or mock infected.
293T cells were transfected with PR8 pDUAL:PB1 (a bidirectional reverse genetics plasmid that encodes the PB1-F2 protein as well as the PB1 protein) or mock transfected, using Lipofectamine 2000 (Thermo Fisher) using cell density and plasmid mass as recommended in the manufacturer's protocol. Cells were lysed in Laemmli buffer at 24 h post-infection or 48 h post-transfection, separated by electrophoresis on 12 % polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked with 5 % milk in PBS-T (phosphate buffered saline with 0.1 % Tween 20) overnight at 4 °C, rinsed in PBS-T then probed with primary antibodies at 1 µg/ml in PBS-T for 1 h at room temperature. Membranes were washed ×3 in PBS-T followed by further incubation with anti-sheep IR680 or IR800 (Thermo Fisher) at 1 in 10,000 in PBS-T, for 1 h at room temperature. Membranes were scanned with a Licor Odyssey CLx Infrared imaging system.

Results

IAV PB1 F2 = 10.54 kDa. Ladder is the Page Ruler Prestained NIR Protein Ladder (Thermo Fisher).

Infected cells



Weak signal detected at ~14kDa for all bleeds at 1µg/ml, but other background bands are observed, depending on the strain.

Recommendation

Antisera could be used at 1 µg/ml for western blot with infected cell lysates, though user validation is recommended due to the presence of other background bands. Expression levels in transfection systems may not be sufficient for a clear signal on Western blot, and user validation is recommended in these cases.

Immunofluorescence:

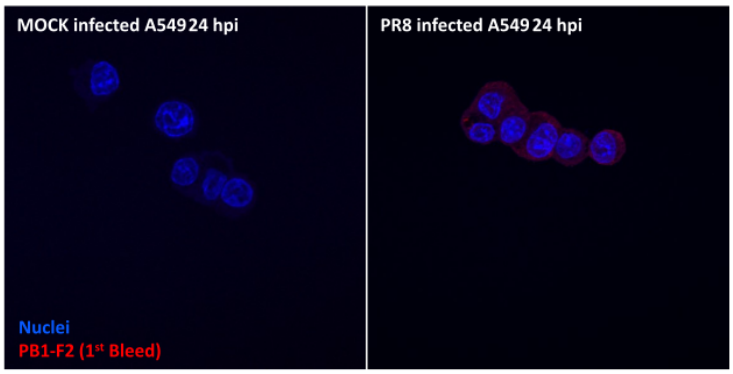
Method

A549 and 293T cells were seeded at 2x10^5 cells/well in 24 well plates containing 13 mm coverslips.
For 293T cells, coverslips were first coated with poly-D-Lysine to promote adhesion.
The day after seeding, A549 cells were infected with PR8 at an MOI of 2 PFU/cell and 293T cells were transfected with as above using Lipofectamine 2000, following the manufacturer's protocol.
Cells were fixed at 24 h post-infection or post-transfection in 4 % formaldehyde in PBS, then permeabilised using 0.2 % Triton-X100 in PBS/2 % fetal bovine serum (FBS) before blocking for up to 1 h in PBS/2 % FBS.
All antibody dilutions were carried out in PBS/2 % FBS. Cells were incubated with primary antibodies at 1:50 for 1 h at room temperature, washed 3 times in PBS/2 % FBS then probed with donkey anti-sheep IgG (H+L) Cross-Adsorbed Secondary antibody, AlexaFluor 647(Invitrogen) at 1:500 and DAPI at 1:1000 for 45 minutes at room temperature.
Cells were washed 3 times in PBS/2 % FBS and once in PBS, excess moisture was removed before mounting coverslips on slides using ProLong Gold Antifade mounting agent (Thermo Fisher).
Mounting agent was allowed to harden overnight before observation using a Zeiss 710 confocal microscope.

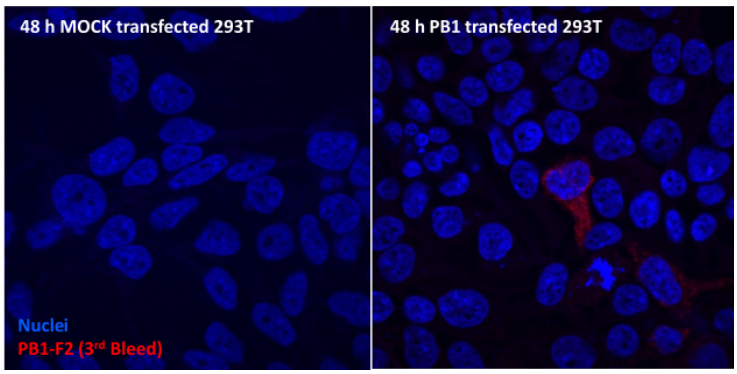
Results

A strong, infection-specific signal was detected for all bleeds in both infection and transfection. Images show 1st bleed antibody at 24 h post-infection and 3rd bleed antibody at 48 h post-transfection; similar results were observed for the other bleeds.

a)



b)



a) Strong signal detected using all bleeds at 1 in 50 dilution in PR8 infected cells.
b) Strong signal detected using all bleeds at 1 in 50 dilution in PB1 transfected cells.

Recommendation

All 5 bleeds are suitable for use in IF of both infected and transfected cells at a 1 in 50 dilution.

PUBLICATIONS:
None to date.

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