MRC PPU
Reagents
and Services

ANTIBODY TESTING RESULTS
Standard Reporting Template

Antibody Name: Anti-IAV-PB1 (18 – 33)

Full Antigen Name: PB1 (Polymerase B1) protein of influenza A virus (IAV) strain A/Puerto Rico/8/1934(H1N1) ('PR8')

Antigen Species: Virus

Antigen: ISTTFPYTGDPPYSHG (residues 18 – 33 of IAV-PB1)

Sheep Number: DA226

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 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 = 86.58 kDa. Ladder is the Page Ruler Prestained NIR Protein Ladder (Thermo Fisher).

No visible bands observed using any of 1st to 5th bleeds anti-PR8-PB1 when used at 1 µg/ml or at 5 µg/ml to probe blots.
This was true for both infected and transfected cell lysates.

Negative results, data not shown

Recommendation

Antisera not suitable for use in immunoblotting under conditions described above.

ELISA:

Method

96 well ELISA plates were coated overnight at room temperature with lysates of PR8 infected or uninfected MDCK cells, with loading amounts normalised by tubulin concentration.
Plates were blocked for 2 h with 5 % milk in PBS-T.
All antibody dilutions were carried out using PBS-T.
Dilution series of the test antisera were added to the plates, prepared in duplicate from an initial concentration of 50 µg/ml using three-fold serial dilutions. Plates were incubated for 2 h at room temperature and washed 3 times in PBS-T.
Donkey Anti-Sheep IgG (H+L), Horseradish Peroxidase (HRP) Conjugate (Life Technologies/Novex) was added to wells at 1 in 20,000 dilution and incubated for 1 h at room temperature.
OPD substrate (Merck) was then added to wells and left to develop for 15 minutes in the dark and at room temperature, before stopping the reaction with 10 % H_2SO_4.
Absorbance (A490) was measured immediately using a Varioskan Lux (Thermo).

Results:

Results were plotted as average absorbance at 490nm at each antibody concentration with results obtained using uninfected lysate coated plates subtracted from results obtained using infected lysate coated plates to correct for any non-specific binding.

No signal above background was detected using any of 1st to 5th bleeds anti-PR8-PB1 using this method.

Negative results, data not shown

Recommendation

Antisera not suitable for use in ELISA using this method

Immunofluorescence:

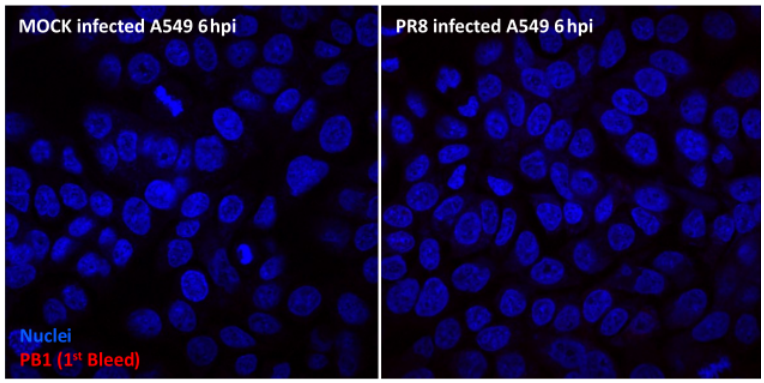
Method

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.

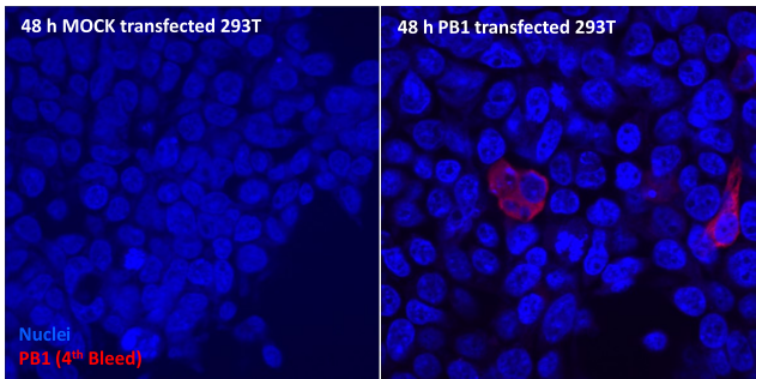
Results

A weak, specific signal was detected for all bleeds in infection. A much stronger, specific signal was detected for all bleeds in transfection. Images show 1st bleed antibody at 6 h post-infection and 4th bleed antibody at 48 h post-transfection; similar results were observed for the other bleeds.

a)



b)



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

Recommendation

All 5 bleeds are suitable for use in IF for detection of PB1 protein in transfected cells at a 1 in 50 dilution

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PUBLICATIONS:
None to date

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