Title	Anti SeV staining protocol
Date Submitted	April 2012
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Adapted from -	
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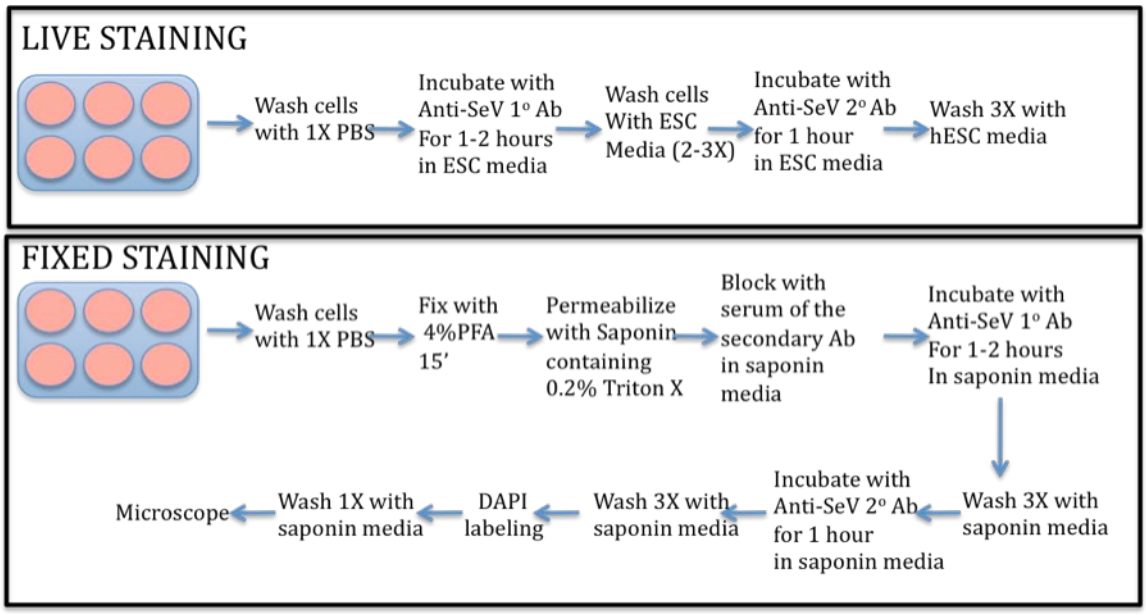
INTRODUCTION

Sendai virus (SeV), also known as murine parainfluenza virus type 1, is a negative sense, single-stranded RNA virus of the paramyxovirus subfamily Paramyxovirinae, genus Respirovirus, members of which primarily infect mammals. The SeV now has been developed as gene transfer vectors for expressing foreign genes to a wide range of mammalian cells and tissues with high efficiency.

FLOWCHART



REAGENT PREPARATION

	Reagent	Company	Catalogue #
1.	10XPBS	Invitrogen	14200166

2.	Saponin	Sigma	S4521-10G
3.	HEPES 1M	Sigma	15630-080
4.	Sodium Azide	Sigma	S2002-25G
5.	Glucose	Sigma	**G7021**
6.	Triton X	Sigma	X100-100ML
7.	PFA	Electron Microscopy	15710
8.	BSA 7.5%	Invitrogen	15260-037
9.	1° Ab - Abcam chicken IgY Abcam	Abcam	33988
10.	goat anti chicken IgY-488	Abcam	ab96951
11.	rabbit SV antiserum	MBL	PD029
12.	donkey anti rabbit AlexaFluor 647	Invitrogen	A21208

REAGENT PREPARATION

SAPONIN MEDIA

	Company	**Working conc**	**Working conc**
PBS-10X	Invitrogen	1X	50ml
Glucose	Sigma	10gm/liter	5gm
BSA (7.5%)	Invitrogen	0.2%	13ml
10%NaN3	Sigma	0.02%	1ml
1M HEPES	Sigma	5mM	2.5ml
Saponin	Sigma	0.5%	0.25gm
Water	Invitrogen	-	433

SAPONIN MEDIA – 0.2% Triton X 100

	Company	**Working conc**	**Working conc**
PBS-10X	Invitrogen	1X	50ml
Glucose	Sigma	10gm/liter	5gm
BSA (7.5%)	Invitrogen	0.2%	13ml
10%NaN3	Sigma	0.02%	1ml
1M HEPES	Sigma	5mM	2.5ml
Saponin	Sigma	0.5%	0.25gm
Water	Invitrogen	-	432
Triton X 100	SIgma	0.2%	1000ul

Wash MEDIA – 0.2% Triton X 100

	Company	**Working conc**	**Working conc**
PBS-10X	Invitrogen	1X	50ml
Glucose	Sigma	10gm/liter	5gm
BSA (7.5%)	Invitrogen	0.2%	13ml
10%NaN3	Sigma	0.02%	1ml
1M HEPES	Sigma	5mM	2.5ml
Water	Invitrogen	-	433

METHOD – LIVE STAINING METHOD

1. Wash the cells with 1X PBS
2. Add 1ml of hESC media and add 5ul of SeV antibody (1° Ab - Abcam chicken IgY Abcam 1:200 dilution) to the cells

OR

3. Add 1ml of hESC media and add 2ul of SeV antibody (1° Ab - rabbit SV antiserum -MBL – 1:500 dilution) to the cells
4. Incubate cells for 1 hour at 37 degrees
5. Wash cells 2-3X with DMEM or hESC media
6. Add 1ml of hESC media and add 2ul of secondary anti-SeV antibody to the cells (1° Ab - Abcam chicken IgY Abcam OR donkey anti rabbit AlexaFluor 647)
7. Incubate for 1 hour at 37 degrees
8. Wash with DMEM media
9. Add hESC media back to the wells
10. Microscope

METHOD – FIXED STAINING METHOD

- Using a 16% % paraformaldehyde, make working solution to 4% PFA in PBS. Make only what you require for the day. Fix the cells for 15' in 4% Paraformaldehide/PBS at room temp.

- Take off the formaldehyde/PBS and wash 2x with PBS at RT with gentle rocking (do atleast once).

- Permeabilize the cells 5' at RT with 0.2 % Triton-X-100 in saponin solution

- wash 2x with PBS at RT with rocking

- to block nonspecific antibody binding add minimum volume of saponin containing 10% secondary antibody serum for 60' at RT (10% donkey serum / 10% goat serum etc.).

- Add primary antibody 1:200 dilution in blocking saponin solution O/N at 4 degrees.

- Aspirate off primary antibody and replace with saponin media for 5-10minutes with gentle rocking. Repeat twice, to give three washes in saponin media

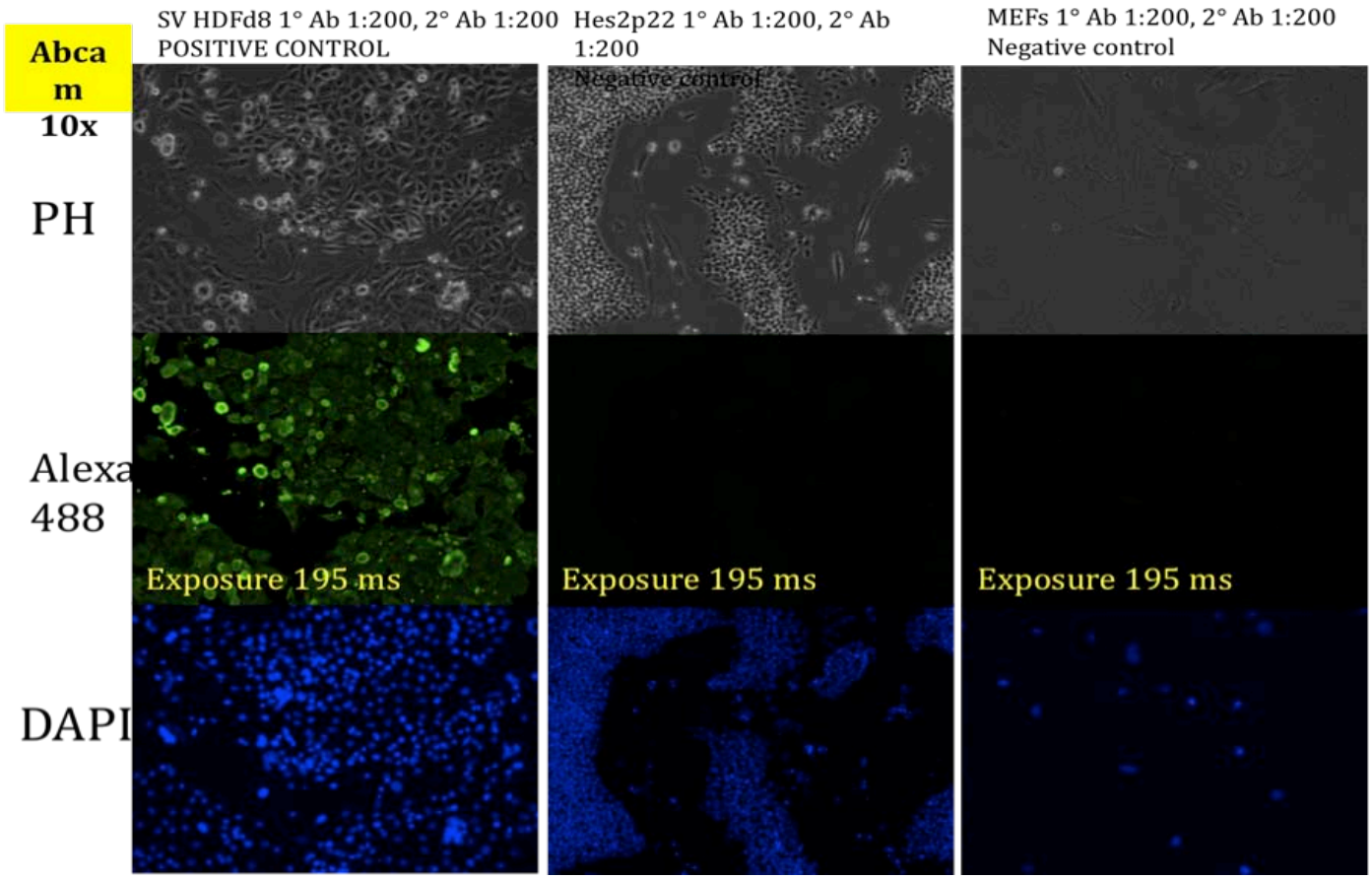
- incubate 60' with 100-200 ul of saponin media containing 1;200 dilution of secondary antibody (without secondary Ab serum).

-Remove secondary antibody and replace with 1 ml of saponin. Give three washes in saponin.

Do the last wash with Dapi (1:1000) dilution for 5-10minutes.
Wash out Dapi using saponin solution (1-2X)

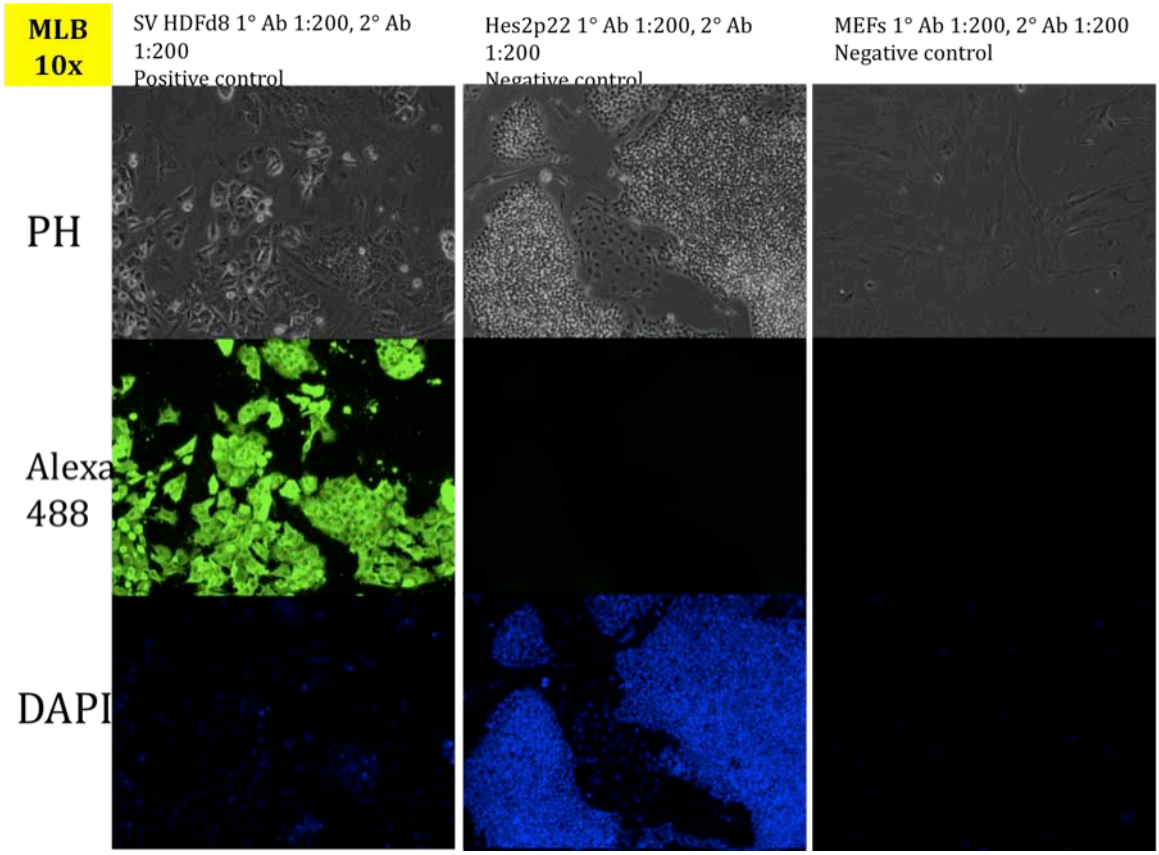
-Fluorescent microscope.

IMAGES



Try to include references to sections and timing in the flow chart

IMAGES



Troubleshooting: If you overexpose, even negative images will appear as positive.

References: Obtained protocols from Abcam and MBL websites.

- Acknowledgements:
  - Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx… (click for more)

- Comments (already in Stembook template):

***There should be an option to print the entire protocol, each section independently, or combinations of sections [1,3,4,5,6].