SYBR Gold Staining for viral enumeration using 13 mm Anodisc 0.02 µm filters

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Abstract

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Protocol

Preparation of filter holders

Step 1.
Cut a 3 ml syringe as a filter funnel:

(Fig 1)

Preparation of filter holders

Step 2.
Take a gasket from 13 mm MILLIPORE filter holder (FISHER, SX0001300).

Fig 2

Preparation of filter holders

Step 3.
Obtain a clamp for 25 filter funnel.

Step 4.
Make dilution of virus prep in 0.02um filtered seawater to a concentration of E+07 particles ml-1.

Prepare working solutions of SYBR-Gold

Step 5.
Thaw the commercial stock of SYBR-Gold in the dark at RT and centrifuge at 3000 rpm for 5 minutes because SYBR-Gold is in DMSO.

DURATION
00:05:00

Prepare working solutions of SYBR-Gold

Step 6.
Centrifuge at 3000 rpm for 5 minutes.

DURATION
00:05:00

NOTES
James Thornton Jr 07 Aug 2015
SYBR-Gold is in DMSO.

Prepare working solutions of SYBR-Gold

Step 7.
Dilute SYBR-Gold in 0.02 µm filtered TE buffer to 100x (10 µl in 990 µl TE buffer).

NOTES
James Thornton Jr 07 Aug 2015
This working stock can be stored at -20°C in small aliquots and re-thaw one time.

Step 8.
Add 1 µl of SYBR working stock in 49 µl 0.02 µm filtered TE buffer in a plastic Petri dish.

NOTES
James Thornton Jr 07 Aug 2015
Can have 4 drops for staining 4 filters in one dish.

Step 9.
Cover the dish by aluminum foil.

NOTES
James Thornton Jr 07 Aug 2015
SYBR is light sensitive.

Step 10.
Set up the filtration unit, connecting it to a vacuum.

NOTES
James Thornton Jr 07 Aug 2015
Set up the vacuum no higher than 5 mm Hg.

Step 11.
Add a few drops of 0.02 µm filtered mQ on the filter base and place a 25 mm 0.2 nitrocellulose filter (the support filter) on top of the water.

Fig 3

Step 12.

Switch on the vacuum, the support filter should be flat on the filter base.

NOTES

James Thornton Jr 07 Aug 2015

This support filter is good for several samples as long as it remains flat and no air bubbles between filter and base.

Step 13.

Place a 13 mm Anodisc filter on the wet nitrocellulose filter.

Fig 4

Preparation of filter holders

Step 14.

Place a gasket on the 13 mm Anodisc filter.

Fig 5

Preparation of filter holders

Step 15.
Place the syringe filter funnel carefully on the gasket and apply the clamp.


Fig 6

Step 16.
Switch on the vacuum and add samples for filtration, leave the vacuum on for 1 more minutes after sample drained completely.

Step 17.
Leave the vacuum on for 1 more minutes after sample drained completely.

DURATION
00:01:00

Step 18.
Take away the clamp and syringe filter funnel while vacuum is on.

Step 19.
Carefully push the filer to the edge of the filter base by tweezers while vacuum is on to remove the filter.

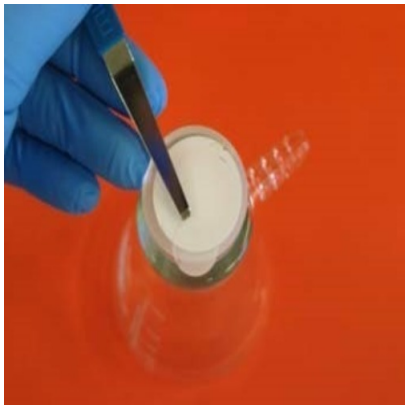
Fig 7

Step 20.
Dry filter membrane on Kimwipes in the dark at RT completely.

NOTES
James Thornton Jr 07 Aug 2015
Better in a paper box.

Step 21.
Remove membrane and place viruses-side up on staining solution in the Petri dish for 15 min, cover the Petri dish by aluminum foil.

DURATION

00:15:00

Step 22.

Cover the Petri dish by aluminum foil.

Step 23.

Dry filter membrane again on Kimwipes in the dark at RT completely.

NOTES

James Thornton Jr 07 Aug 2015

Better in a paper box.

Step 24.

Pipet 10 µl antifade solution on a microscope slide and place the stained filter membrane on top of it. Pipet 10 µl antifade solution on a coverslid and carefully place it on the filter to avoid bubbles.

Step 25.

Step 26.

Pipet 10 µl antifade solution on a coverslid and carefully place it on the filter to avoid bubbles.

Step 27.

Place slide at –20°C to enhance fluorescence.

Step 28.

Read slides using 100x oil immersion objective and inverted fluorescent microscope.