SYBR Gold Staining for Viral Enumeration (Case 1)

Li Deng

Abstract

Case 1: Quick check of viral concentration. Use this protocol when you can count your samples in a few days.

For when long term storage of slide is required, see Case 2.

Citation: Li Deng SYBR Gold Staining for Viral Enumeration (Case 1). **protocols.io**
dx.doi.org/10.17504/protocols.io.c69zh5
Published: 04 Jan 2016

Protocol

Step 1.
Make dilution of virus prep in 0.02 µm filtered seawater to a concentration of E+07 particles ml^{-1}

Step 2.
Prepare working solutions of SYBR Gold

PROTOCOL

. SYBR Gold working solutions

CONTACT: VERVE Team

Step 2.1.
Thaw the commercial stock of SYBR Gold in the dark at RT

Step 2.2.
Centrifuge at 3000 rpm for 5 minutes

DURATION
00:05:00

NOTES
VERVE Team 24 Jun 2015
Because SYBR Gold is in DMSO.

Step 2.3.
Dilute SYBR Gold in 0.02 µm filtered TE buffer to 100x (10 µl in 990 µl TE buffer)

Step 2.4.
0.02 µm filter the diluted SYBR (100x)

NOTES
VERVE Team 24 Jun 2015
This working stock can be stored at -20°C and re-thawed one time.

Step 3.
Add 2 µl of SYBR working stock in 98 µl 0.02 µm filtered mQ in a plastic Petri dish, 4 drops in one dish

Step 4.
Cover the dish by aluminum foil

NOTES
VERVE Team 24 Jun 2015
SYBR is light sensitive.

Step 5.
Set up the filtration unit, connecting it to a vacuum

NOTES
VERVE Team 24 Jun 2015
Set up the vacuum no higher than 5 mm Hg.

Step 6.
Add a few drops of 0.02 µm filtered mQ on the filter base

Step 7.
Place a 0.2 nitrocellulose filter (the support filter) on top of the water

Step 8.
Switch on the vacuum, the support filter should be flat on the filter base

NOTES
VERVE Team 11 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 9.
Add a few drops of 0.02 µm filtered mQ on the support filter

Step 10.
Switch on the vacuum to pull the water through

Step 11.
Apply a 0.02 µm Anodisc filter over the support filter

NOTES
VERVE Team 11 Aug 2015
Make sure no air bubbles between filters.

Step 12.
Apply the filter tower and clamp while vacuum is on

Step 13.
Switch off the vacuum and add viral samples

Step 14.
Switch on the vacuum and wash filter set with 1 ml of 0.02 µm filtered mQ

AMOUNT
1 ml Additional info:

Step 15.
Remove the filter while the vacuum is still on

Step 16.
Rinse tower in 1L Q-water in between samples

Step 17.
Blot onto paper towel to dry

Step 18.
Dry filter membrane on Kimwipes at RT completely

Step 19.
Remove membrane and place viruses side up on staining solution in the Petri dish for 15 minutes (cover with aluminum foil)

DURATION
00:15:00

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

NOTES
VERVE Team 26 Jun 2015
Better in a paper box.

Step 21.
Pipet 20 µl antifade solution on a microscope slide

Step 22.
Place the stained filter membrane on top of it

Step 23.
Pipet 30 µl antifade solution on a cover slide

Step 24.
Carefully place it on the filter to avoid bubbles

Step 25.
Place slide at -20°C to enhance fluorescence

Step 26.
Read slides using 100x oil immersion objective and inverted fluorescent microscope