# SYBR Gold Staining for Viral Enumeration (Case 2)

**Li Deng**

## Abstract

Case 2: Fix samples. Use this protocol when long term storage of slide is required.

For when you can count your samples in a few days, see Case 1.

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## Protocol

**Step 1.**
Prepare buffer glutaraldehyde:

PROTOCOL

**. Buffered glutaraldehyde**

CONTACT: Bonnie Poulos
**Step 1.1.**
Prepare solution A and solution B as found in guidelines

**Step 1.2.**
Prepare the phosphate buffere by adding 15.25 ml solution A and 9.75 ml solution B in 25 ml 0.02 µm filtered mQ

NOTES
**Bonnie Poulos** 24 Jun 2015
= 50 ml buffer

**Step 1.3.**
Add 40 ml of 25% glutaraldehyde to 10 ml phosphate buffer for a 20% buffered glutaraldehyde solution

NOTES
**Bonnie Poulos** 30 Nov 2015
This is most often used for transmission electron microscopy so purchase a TEM-grade glutaraldehyde (from a vendor such as Ted Pella) for preparing this buffered reagent

**Step 1.4.**
Adjust pH to 7 with NaOH

**Step 1.5.**
Store this 20% buffered glutaraldehyde at 4 °C in the dark

**Step 2.**
Make dilution of virus prep in 0.02 µm filtered seawater to a concentration of E+07 particles $ml^{-1}$

**Step 3.**
Fix viral sample with 20% buffered glut to a final concentration of 0.5%

**Step 4.**
Incubate at 4°C for 30 min.

DURATION
00:30:00

**Step 5.**
Prepare working solutions of SYBR Gold

PROTOCOL

**. SYBR Gold working solutions**

CONTACT: VERVE Team

**Step 5.1.**
Thaw the commercial stock of SYBR Gold in the dark at RT

**Step 5.2.**
Centrifuge at 3000 rpm for 5 minutes

DURATION
00:05:00

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

**Step 5.3.**
Dilute SYBR Gold in 0.02 µm filtered TE buffer to 100x (10 µl in 990 µl TE buffer)

**Step 5.4.**
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 6.**
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 7.**
Cover the dish by aluminum foil

NOTES
**VERVE Team** 24 Jun 2015
SYBR is light sensitive.

**Step 8.**
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 9.**
Add a few drops of 0.02 µm filtered mQ on the filter base

**Step 10.**

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

**Step 11.**
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 12.**
Add a few drops of 0.02 µm filtered mQ on the support filter

**Step 13.**
Switch on the vacuum to pull the water through

**Step 14.**
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 15.**
Apply the filter tower and clamp while vacuum is on

**Step 16.**
Switch off the vacuum and add viral samples

**Step 17.**
Switch on the vacuum and wash filter set with 1 ml of 0.02 µm filtered mQ

AMOUNT
1 ml Additional info:

**Step 18.**
Remove the filter while the vacuum is still on

**Step 19.**
Rinse tower in 1L Q-water in between samples

**Step 20.**
Blot onto paper towel to dry

**Step 21.**
Dry filter membrane on Kimwipes at RT completely

**Step 22.**
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 23.**
Dry filter membrane again on Kimwipes in the **dark** at RT completely

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

**Step 24.**
Pipet 20 µl antifade solution on a microscope slide

**Step 25.**
Place the stained filter membrane on top of it

**Step 26.**
Pipet 30 µl antifade solution on a cover slide

**Step 27.**
Carefully place it on the filter to avoid bubbles

**Step 28.**
Place slide at -20°C to enhance fluorescence

**Step 29.**
Read slides using 100x oil immersion objective and inverted fluorescent microscope