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SYBR Gold staining of viral-like particles on Anodiscs

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Aditi K. Narayanan¹, Alon Philosof¹

¹California Institute of Technology

Aditi K. Narayanan: ORCID: 0000-0003-0627-1859

Alon Philosof: ORCID: 0000-0003-2684-8678

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California Institute of Technology

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for SYBR-Gold staining of liquid suspensions of viral-like particles on aluminum oxide (Anodisc) filters. Adapted from Noble and Fuhrman 1998 (doi:10.3354/ame014113) and dx.doi.org/10.17504/protocols.io.c69zh5 from the Sullivan Lab

Aditi K. Narayanan and Alon Philosof

- 1 Adapted from Noble and Fuhrman 1998 (doi:10.3354/ame014113) and [dx.doi.org/10.17504/protocols.io.c69zh5](https://doi.org/10.17504/protocols.io.c69zh5) from the Sullivan Lab

You should have your cell-free suspension of viruses ready (i.e. already filtered through a 0.2µm pore size membrane to remove cells and debris) before beginning this protocol.

Materials

- 2
 1. SYBR Gold Nucleic Acid Gel Stain (10,000X Concentrate in DMSO), Invitrogen (now ThermoFisherScientific) Cat.#S11494
 2. VectaShield antifade mounting medium, Vector Laboratories #H-1000-10
 3. Sterile virus-free water (autoclaved or, preferably, 0.02µm filtered through Whatman Anotop 0.02µm filter, product #6809-2102)
 4. Sterile virus-free 1X PBS (autoclaved or, preferably, 0.02µm filtered)
 5. Whatman Anodisc, 0.02µm pore size and 25mm diameter, with support ring, Cytiva #6809-6002 (EXPENSIVE! FRAGILE! Use carefully)
 6. Any hydrophilic 5µm pore size filter membranes (e.g. made from PES), 25mm diameter
 7. Plain glass microscope slides and coverslips
 8. Kimwipes
 9. Filter Forceps
 10. 25mm diameter vacuum filter holder
 11. Sterile Petri dishes

Prepare SYBR Gold solution

- 3 Dilute 5µL of the 10,000X SYBR Gold into 45µL of virus-free 1X PBS for a final concentration of 1000x. Keep in the dark.

We recommend aliquoting the 10,000X solution as soon as it arrives into 200µL PCR tubes (5µL per tube). Store the aliquots at -20°C and take out individual tubes as needed. This avoids repeated freezing and thawing of the main stock.

- 4 For each sample, mix 2.5µL of the 1000X solution with 97.5µL virus-free water. Store at 4°C and protected from light.

The remaining 1000x solution will keep in the 4°C for about a week. For sensitive or highly quantitative projects, we suggest making a fresh solution every time.

- 5 For each sample, pipette 100µL of the 25X working solution onto a Petri dish, ensuring that the surface tension on the drops remains intact. Do not let each of the 100µL drops

run into the edges of the plate. Keep the dishes in the dark.

Filter samples onto Anodiscs

- 6 Set up your vacuum pump and filter flask. Dampen the filter holder with MilliQ water or virus-free water and put into the top of the flask.

- 7 Use the filter forceps to place a 5µm backing filter onto the filter holder. Place an Anodisc on top of it, dampening the top of the backing filter beforehand if necessary.



Ensure that the Anodisc is facing the right way up! They come packed face up, but in case you lose track, look for a thin border of the plastic ring on TOP of the disc. If the plastic ring appears to be under the disc, you have it upside down.

- 8 You can proceed in one of two ways:

1. If you do not care about the actual number of viruses and just want a broad sense of presence and density, you can use a pipette to carefully drop your sample onto the anodisc while the vacuum is running.

This has the advantage of letting you put multiple samples on a single disc if you pipette carefully within an area. You can also use a PAP pen to divide the disc into sections (e.g. Vector Laboratories ImmEdge Hydrophobic Barrier PAP Pen #H-4000).

2. If you do care about being quantitative, attach a glass reservoir to the top of the vacuum flask with a pair of clamps. The order, from the bottom, is thus: Filter holder, 5µm backing filter, 0.02µm Anodisc, and glass reservoir. You can then pour your sample into the reservoir and allow it to evenly spread across the anodisc before turning on the vacuum.

This will allow you to perform accurate VLP counts.

- 9 Once all the viral suspension has been deposited onto the Anodisc (i.e. no liquid is visible on the disc), turn off the pump and CAREFULLY remove the disc from the filter tower. Allow to air dry, either in the grasp of the forceps or on a dry slide or Petri dish.

You may also want to wash the sample on the disc by adding additional virus-free water to the reservoir and allowing it to filter through. Depending on your sample, this may help reduce background noise from the eventual SYBR-Gold staining.

- 10 Repeat 7-9 for all remaining samples. We highly recommend filtering the virus free water and 1X PBS onto their own Anodiscs as negative controls.



Staining and mounting

- 11 Place each sample-coated Anodisc FACE UP onto a 100 μ L droplet of the 25X SYBR Gold solution.

It is critical that you stain with the surface of the disc facing up, allowing the SYBR solution to seep up through the underside. In our experience, this results in a cleaner signal.

- 12 Cover the Petri dishes and incubate them in the dark for 15 minutes  00:15:00
(coating the lids in aluminum foil works well here).

- 13 At the end of the incubation period, gently lift each Anodisc up by its edge. This may take some practice due to surface tension, and is the most likely step in which the discs crack.

- 14 Gently wick excess stain from the bottom of the discs using a Kimwipe. Place the disc onto a microscope slide and allow it to dry completely. This will be the slide you use for the microscopy, so we suggest labeling it in advance.

- 15 Once dry, place a drop of Vectashield on the disc and mount with a coverslip. When visualizing, use a 100X oil immersion objective. The excitation peak for SYBR Gold is approximately 496nm.

Protocol references

Noble and Fuhrman 1998 (doi:10.3354/ame014113)

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