Sep 12, 2021

Epifluorescence-Microscopy-VLPs

Josue L Castro-Mejia[1]

[1]Department of Food Science, University of Copenhagen

1 Works for me Share

dx.doi.org/10.17504/protocols.io.bx6cpraw

FOOD Micro UCPH

Josue L Castro-Mejia

ABSTRACT

Epifluorescence Microscopy for Viral-like Particles
-Virome Studies-

DOI

dx.doi.org/10.17504/protocols.io.bx6cpraw

DOCUMENT CITATION

Josue L Castro-Mejia 2021. Epifluorescence-Microscopy-VLPs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bx6cpraw

LICENSE

This is an open access document distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Sep 12, 2021

LAST MODIFIED

Sep 12, 2021

DOCUMENT INTEGER ID

53156

ABSTRACT

Epifluorescence Microscopy for Viral-like Particles
-Virome Studies-

Reagents

- SYBR Gold
- Glycerol
- PBS: (0.05M Na2HPO4, 0.85%(wt/vol) NaCl (pH 7.5)
- 0.02µm filtered-autoclaved MilliQwater

Equipment

- 0.02µm filter whatman Al_2O_3 (Whatman: 6809-6002)
- Microscope glass slides
- Glass coverslips

Citation: Josue L Castro-Mejia (09/12/2021). Epifluorescence-Microscopy-VLPs. https://dx.doi.org/10.17504/protocols.io.bx6cpraw

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

- Falcon tubes
- Petri dishes
- Wipes
- Swinnex Filter holder (Millipore: SX0002500)
- 5ml syringe

Reagents Setup

- **50% glycerol/50% PBS (1:1) (Anti-fading/mounting Solution)**
  Sterilized through 0.02µm membrane, autoclaved and stored in falcon tubes at -20°C.
- **SYBR Gold solution (1000x)**
  975µl of 0.02µm filter-autoclaved MilliQwater
  25 µl of SYBR Gold 1000x (prepared)
  Prepare solution fresh-before use

Protocol

Filter Preparation

1. Filter holders (Swinnex) must be autoclaved or immersed in 70% EtOH overnight.

2. Subsequently, place the 0.02µm anodisc on top of the filter, close the lid and the filter is ready to use (**REMEMBER** to use filter forceps and wear gloves to avoid contamination).

Dilution of phages

3. Dilute 5 µl of CsCl gradient sample in 2.5 ml of 0.02µm filtered-autoclaved MilliQwater.

4. Using a 5 ml syringe, filter the diluted samples. When filtrating, be gentle with the pressure applied to avoid damage of the viral-like particles.

5. Carefully, remove anodisc (from holder) using filter forceps and place it on a clean Kimwipe to blot the back of the Anodisc filter.

6. Anodisc should be dried, by placing it on a new Kimwipe in a dark box or bench drawer. When the filter is dried, it will not be translucent (10-15 min).

Preparation of SYBR Gold for staining (while filters are drying)

7. Enumerate petri dishes according to your samples/treatments.

8. Dispense 100µl droplet of the SYBR Gold solution (1:38) on a Petri Dish.

9. Once anodiscs are dried, place one of them onto a 100 µl droplet and stain for 25-30 min on **DARK**conditions, e.g. in a dark box or bench drawer.

10 .After staining, carefully pick up the anodiscs and repeat drying procedures from steps 6 and 7.

11. Thereafter, dried anodiscs are mounted on labeled glass slides. Add 15 µl of mounting medium (ANTI-FADING solution) onto the glass to facilitate the filter positioning. Subsequently, 15 µl of mounting medium (ANTI-FADING solution) are also deposited on top of the anodisc to facilitate attachment of the glass coverslip (Noble & Fuhrman, 1998).

12. Sample is ready to be observed though the FM.

Citation: Josue L Castro-Mejia (09/12/2021). Epifluorescence-Microscopy-VLPs. https://dx.doi.org/10.17504/protocols.io.bx6cpraw

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited