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 We are still developing and optimizing this protocol

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 41803

Oceanit Lateral Flow Assay (LFA) Protocol

proposals¹, Dexter Poon¹

¹Oceanit Laboratories, Inc.

Dexter Poon: XPrize submission

proposals

ABSTRACT

This experiment uses infectious WA1 strain of SARS-CoV2 and is conducted in a qualified BSL3 facility at the University of Hawaii John A Burns School of Medicine (Honolulu, Hawaii).

MATERIALS

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RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #EN0531**

Triton X-100 **VWR Scientific Catalog #9002-93-1**

BSA **Sigma Aldrich Catalog #A2153**

Gold Nanospheres - Bare (Citrate) 80nm **Nanocomposix Catalog # AUCN80**

TBSTween **Sigma Aldrich Catalog #9039-10PAK**

Sample Pad **Cytiva Catalog #8134-2250**

Oceanit Lateral Flow Assay (LFA) Protocol

1 Prepare Serial Dilution

This experiment uses infectious WA1 strain of SARS-CoV2 and is conducted in a qualified BSL3 facility at the University of Hawaii John A Burns School of Medicine (Honolulu, Hawaii).

2 Aliquot diluent solution into centrifuge tubes

Diluent solution is cell growth media supplemented with 10% FBS.

3 Prepare lysis buffer with LFA capture molecule

Lysis buffer containing LFA capture molecule is Oceanit's proprietary formulation.

4 Virus stock (4×10^7 pfu/mL)

Virus stock 10-fold serial dilutions.

5 10-fold serial dilutions and control tubes

a. Tube 1 – Neat (4×10^7 pfu/mL); pfu = plaque forming units

b. Tube 2 – 10^{-1}

c. Tube 3 – 10^{-2}

d. Tube 4 – 10^{-3}

e. Tube 5 – 10^{-4}

f. Tube 6 – 10^{-5}

g. Tube 7 – 10^{-6}

h. Tube 8 – 10^{-7}

i. Control – no virus

6 Add lysis buffer to dilutions

Add prepared lysis buffer to labeled individual LFA cassettes. Test and control lines are Oceanit's proprietary molecules.

7 Incubate 5 minutes

8 Add to labeled individual LFA cassettes.

Add 250uL of the corresponding sample to individual LFA cassettes.

9 Record results with photography:

A.Amount:

B.Concentration: Virus stock and dilution range from 4×10^7 pfu/mL to 0 virus added.

C.Temperature: Virus stocks are stored at -80C; assay is performed at room temperature.

D.Duration of the experiment: 1 hour elapsed time.

E.Equipment: BSL3 containment, Class II biological safety cabinet, autoclave, pipette and disposable tips, disposable tubes, dedicated camera.

F.Reagents: Vero E6 cell culture growth media supplemented with 10% FBS, Oceanit lysis buffer + capture molecule (proprietary), LFA embedded test and control line molecules (proprietary)