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Live Virus EV-D68 - on RD cells - Antiviral Screening Assay

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We use this protocol and it's working

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Abstract

The following is a protocol for live virus antiviral screening against EV-D68 in vitro on Rhabdomyosarcoma (RD) cells. RD Cells are prophylactically treated with antiviral candidates that have been serially diluted to investigate activity. IC50 values are generated via analysis of MTT/MTS data as a readout of CPE (cytopathic effect).
Each live virus screening assay is accompanied by a matching cytotoxicity screening assay in uninfected cells to investigate drug toxicity.

Guidelines

4. Adding Virus:
   a. Prepare virus to appropriate dilution in ASSAY MEDIUM
      a. EV-D68: Prepare a 100 TCID50/mL (adding 100 µL/well results in 10 TCID50/well and thus with 20,000 cells and MOI = 0.0004 TCID50/cell
         i. For the current screening stock which is 1.00×10^8 TCID50/mL a 1/5×10^5 predilution is needed.
   b. Add 100 µL of virus preparation to C1-10

5. Incubate plates (35°C / 5% CO2)

6. On day 4 determine cell viability using MTS (see Jochmans D et al. J Virol Methods. 2012 Aug;183(2):176-9. doi: 10.1016/j.jviromet.2012.04.011).

For toxicity measurement the same protocol is applied but without virus.

Materials

- DMEM (gibco cat no 41965-039)
- Heat-inactivated FCS
- Pen-Strep (10,000 U/mL) (Gibco cat no 15140-148)
- Trypsin 0.25%/trypsine/EDTA
- DPBS
- T150 bottle
- 96-well plate (example Greiner Bio One 655090 or Falcon)

Safety warnings

Please be sure to wear proper Personal Protective Equipment (PPE) while performing this experiment.

Note

1 This protocol can be used for anti virus assay and the equivalent Cytotoxicity assay where no virus is added.
For the cytotoxicity assay, do not add the virus in the later stages

Cell Culture (1/10 split, twice each week)

2 RD Cells are propagated in GROWTH MEDIUM which is prepared by supplementing DMEM (gibco cat no 41965-039) with 2% v/v heat-inactivated FCS and 1% Pen-Strep (10,000 U/mL)(Gibco cat no 15140-148). Cells are cultured at 37°C, 5%CO2 in T150 bottle in 35 mL GROWTH MEDIUM and split 1/10 twice a week. Detachment is performed by washing the monolayer 1x with DPBS, cover cell layer 1 minute with 5 mL trypsine 0.25%trypsine/EDTA at room temperature, remove 4.5 mL and incubate 3 minute at 37°C.

Antiviral Assay

3 ASSAY MEDIUM is prepared by supplementing DMEM (gibco cat no 41965-039) with 2% v/v heat-inactivated FCS, 1% Pen-Strep (10,000 U/mL)(Gibco cat no 15140-148).

4 Prepare cell suspension:

4.1 start with confluent T150 bottle

4.2 wash monolayer with DPBS

4.3 add 5 mL trypsine 0.25%trypsine/EDTA, cover the cell layer by placing the bottle horizontally, incubate for 1 min at room temperature, remove 4.5 mL of the trypsine solution

4.4 incubate 15 minutes at 37°C

4.5 resuspended in 10 mL ASSAY MEDIUM

4.6 count

4.7 resuspend at 25,000 cells/100 µL in ASSAY MEDIUM

4.8 seed 100 µL of cell suspension to each well of a 96w plate (example Greiner Bio One 655090 or Falcon)

4.9 in case of insufficient cells: medium instead of cells can be added to border wells

5 Incubate plates overnight (37°C / 5%CO2)

6 Adding Compound:

6.1 Add 100 µL ASSAY MEDIUM to C11 and C12

6.2 Add 50 µL ASSAY MEDIUM to C2

6.3 Add compound to C2 row B-G:

6.4 For stock 10 mM to 100 µM final start conc. you need to add 3 µL. With stock 10 mM and 10 µM final start conc. you prepare a 1/10 predilution in ASSAY MEDIUM (for example mix 10 µL 10 mM with 90 µL in C1 and add 3 µL to C2).

6.5 Dilute 1/3 over the plate (transfer 50 µL) from C2-9 – change tips in C4 and C7

7 Adding Virus:

7.1 Prepare virus to appropriate dilution in ASSAY MEDIUM

7.2 EV-D68: Prepare a 100 TCID50/mL (adding 100 µL/well results in 10 TCID50/well and thus with 20,000 cells and MOI = 0.0004 TCID50/cell

7.3 For the current screening stock which is 1.00×10^8 TCID50/mL a $1/5\times10^5$ predilution is needed.

7.4 Add 100 µL of virus preparation to C1-10

8 Incubate plates (35°C / 5% CO2)

9 On day 4 determine cell viability using MTS (see Jochmans D et al. J Virol Methods. 2012 Aug;183(2):176-9. doi: 10.1016/j.jviromet.2012.04.011).

10 For toxicity measurement the same protocol is applied but without virus.

Protocol references

A novel method for high-throughput screening to quantify antiviral activity against viruses that induce limited CPE

Jochmans D et al. J Virol Methods. 2012 Aug;183(2):176-9. doi: 10.1016/j.jviromet.2012.04.011.

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