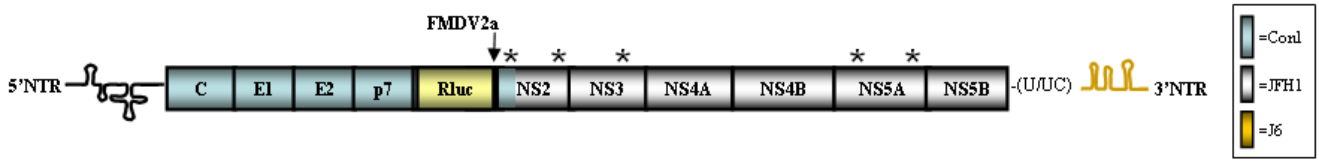
mCon1/JFHRluc2(j5.1; adapted)

Virus Specifications:

Description:
mCon1/JFHRluc2(j5.1; adapted) is a genotype 1b/2a chimeric reporter virus in which the HCV IRES drives translation of a *Renilla* luciferase-containing HCV polyprotein that is cleaved by cellular and viral proteases to produce the individual HCV proteins. The *Renilla* luciferase is fused C-terminal to the p7-NS2 cleavage site, and is cleaved from the HCV polyprotein by virtue its fusion with the foot-and-mouth disease virus 2a peptide coding sequence (FMDV2a). This virus contains five adaptive mutations that were identified after passaging the parental virus in cell culture to generate a variant that produces high titers.



Genotype: 1b/2a
Strains: Con1—core through the N-terminal region of NS2
JFH1--5'NTR; N-terminal region of NS2 to NS5B
J6--3'NTR[1]
Location of chimeric junction: between the first and second predicted transmembrane domains of NS2[2]
Reporter: *Renilla* luciferase
Configuration: monocistronic
Reporter location: C-terminal to the p7-NS2 junction
Adaptive mutations: one within the Con1 region of NS2, one within the JFH1 region of NS2, one within NS3, two within NS5A[3]; adaptive mutations are indicated in the above figure with an asterisk.
Maximal titer range: low 10^3 to low 10^4 $TCID_{50}$/ml
Time point post-electroporation at which maximal titers are achieved: 48 hours
Plasmid designation: APP152
Virus designation: APV152

Virus Production:

Description: Due to the error-prone nature of the HCV RNA-dependent RNA polymerase, it is recommended that stocks be generated from *in vitro* transcribed (IVT) RNA generated from the cloned viral genome. Virus is produced following electroporation of IVT RNA into the highly permissive human hepatoma cell line Huh7.5. The Huh7.5 cell line was generated by curing a stably selected replicon-containing cell line[4], and are the industry-standard for the production of high titer culture-produced HCV.

Huh7.5 cell line designation: APC49
Cell yield from a confluent T150 flask: 6 – 8 x 10^6 cells
Growth conditions: 5% CO_2 and 37°C
% viability post-freeze: > 90%

[1] Differences between the J6 and JFH1 3'NTRs lie within the variable region; side-by-side comparison of a construct containing the J6 3'NTR versus the JFH1 3'NTR revealed no titer differences
[2] Pietschmann *et al, PNAS* (2006) 103: 7408-13
[3] Lacks adaptive mutations in E1 and E2
[4] Blight KJ *et al, J Virol* (2002), 76: 13001-14

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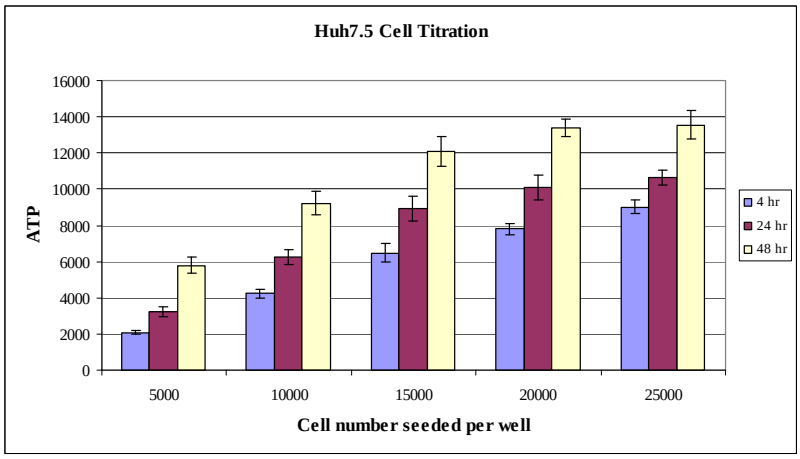

Figure 1. Huh7.5 cell titration growth curve. The indicated number of Huh7.5 cells was seeded into 96-well plates at time = 0. To quantify the relative number of viable cells, intracellular ATP levels were quantified 4, 24, and 48 hours post-plating.

Recommended HCV Antiviral Assay:

1) Infection assay to determine efficacy (EC_{50}) and viability (CC_{50}):

EC_{50} assay: Test Article (at varying concentrations) and HCV virus that expresses *Renilla* luciferase are added simultaneously to Huh7.5 human hepatoma cells**.** *Renilla* luciferase levels are quantified 48 hours after Test Article/virus addition using the Promega *Renilla* Luciferase Assay System to determine the level of viral replication inhibition.

CC_{50} assay: Test Article (at varying concentrations) is added to Huh7.5 human hepatoma cells in the absence of HCV virus. Intracellular ATP levels are quantified 48 hours after Test Article addition using the Promega Cell Titer-Glo^{R} Luminescent Cell Viability Assay System.

Raw data format EC_{50}: *Renilla* luciferase expression in Light Counts Per Second (LCPS)
Raw data dormat CC_{50}: Luminescence measured in LCPS
Calculated data format: Percent Inhibition relative to solvent vehicle-treated control
Maximum number of EC_{50} and CC_{50} values per 96-well plate: four
Signal-to-noise ratio: >500

<u>Efficacy Determination for HCV Inhibitors</u>:

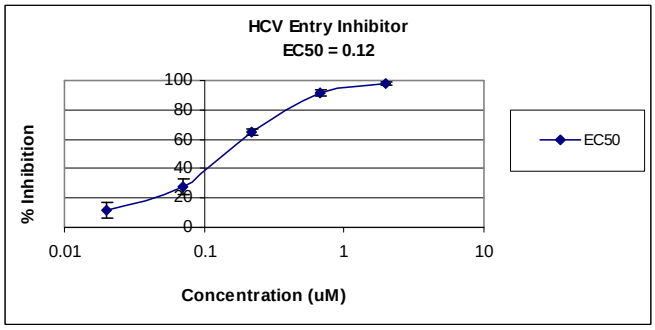

Figure 2. EC_{50} determination in APV152 infection assay in which the Test Article is a protein-based HCV entry inhibitor. Cells were seeded 24 hours prior to addition of Test Article and APV152 at MOI = 0.1. The assay proceeded for 48 hours, at which time *Renilla* luciferase activity was measured. EC_{50} = 0.12 ug/ml

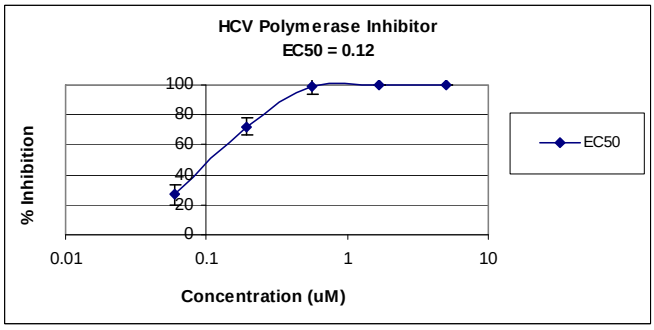

Figure 3. EC_{50} determination in APV152 infection assay in which the Test Article is an HCV polymerase inhibitor. The assay was performed as described in Figure 2. EC_{50} = 0.12 uM, CC_{50} >25 uM (data not shown)

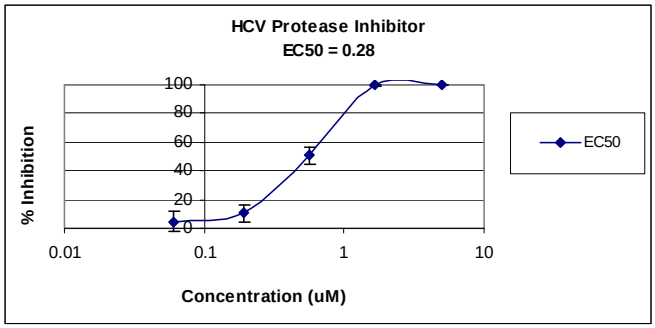

Figure 4. EC_{50} determination in APV152 infection assay in which the Test Article is an HCV protease inhibitor. The assay was performed as described in Figure 2. EC_{50} = 0.28 uM, CC_{50} > 25 uM (data not shown)

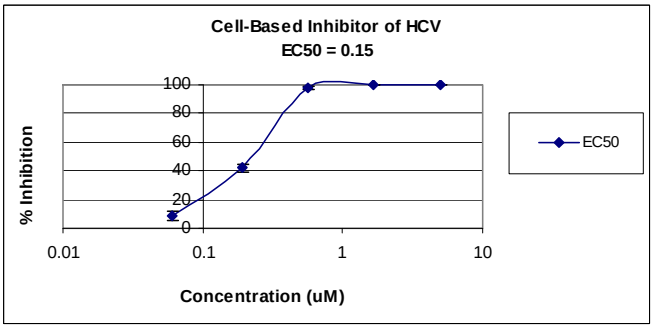

Figure 5. EC_{50} determination in APV152 infection assay in which the Test Article is an HCV cell-based inhibitor . The assay was performed as described in Figure 2. EC_{50} = 0.15 uM, CC_{50} > 25 uM (data not shown)