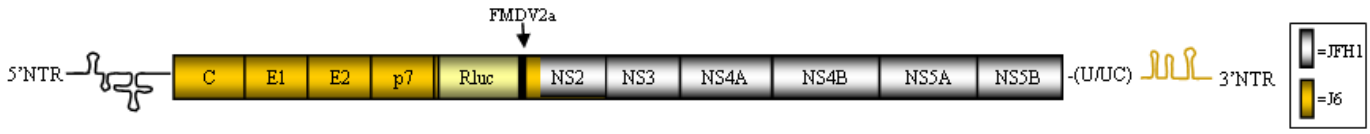
mJ6/JFHRluc2(j5.1)

Virus Specifications:

Description:
mJ6/JFHRluc2(j5.1) is a genotype 2a/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 at C-terminus by virtue its C-terminal fusion with the foot-and-mouth disease virus 2a peptide coding sequence (FMDV2a).



Genotype: 2a/2a
Strains: J6--core through the N-terminal region of NS2; 3'NTR[1]
JFH1--5'NTR; N-terminal region of NS2 to NS5B
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: none
Maximal titer range: low 10^3 to low 10^4 $TCID_{50}$/ml
Time point post-electroporation at which maximal titers are achieved: 48-72 hours
Plasmid designation: APP24
Virus designation: APV24

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[3], and are the industry-standard for the production of cell high titer culture-produced HCV.

Huh7.5 cell line designation: APC166
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 this 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] Blight KJ *et al, J Virol* (2002), 76: 13001-14

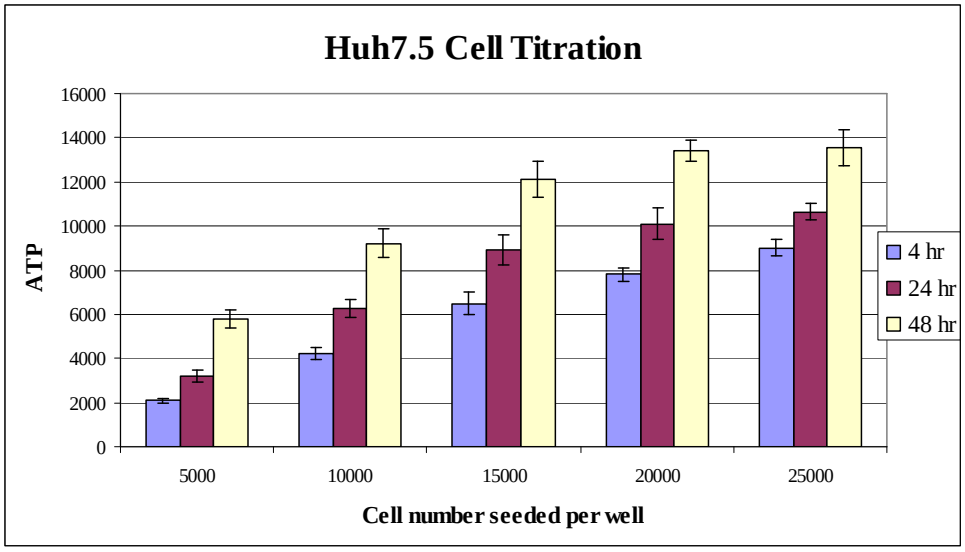


Figure 1. Huh7.5 cell titration growth curve. The indicated number of Huh7.5 cells were seeded into 96-well plates at time = 0. To quantify the total relative number of viable cells, intracellular ATP levels were quantified 4, 24, and 48 hours post-plating.

HCV Antiviral Assays:

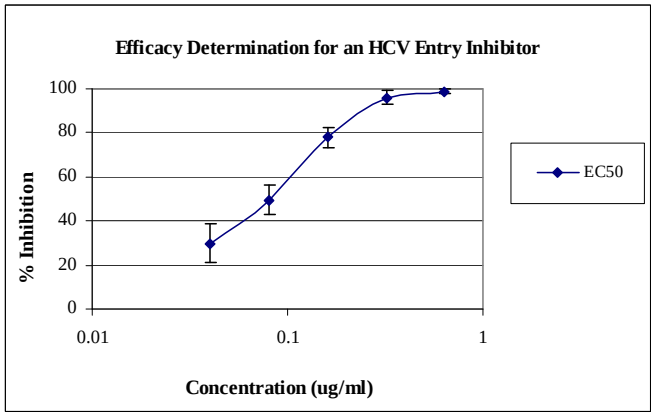
1) Infection assay to determine efficacy (EC_{50}) and viability (CC_{50}):

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: 4
Signal-to-noise ratio: >500

HCV Inhibitor Performance:



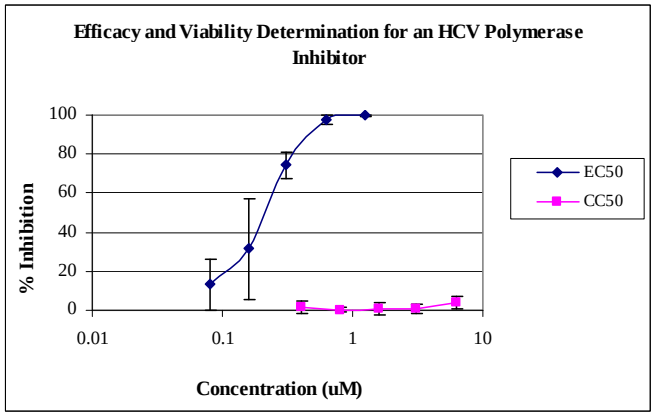


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Figure 3. EC_{50} determination in APV24 infection assay in which the Test Article is an HCV polymerase inhibitor (nucleoside analog). The assayed was performed as described in Figure 2. EC_{50} = 0.18 uM, CC_{50}>6.3 uM

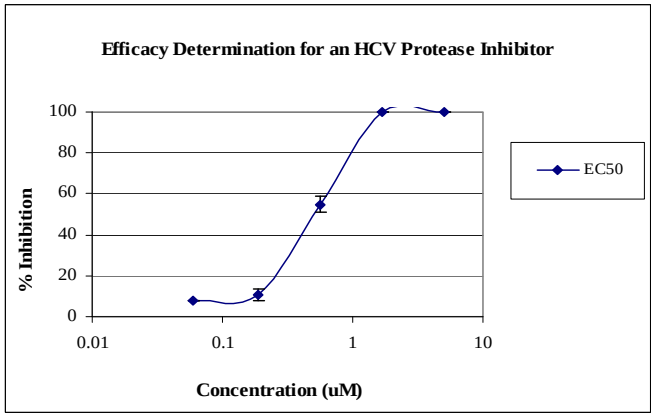

Figure 4. EC_{50} determination in APV24 infection assay in which the Test Article is an HCV protease inhibitor. The assayed was performed as described in Figure 2. EC_{50} = 0.26 uM

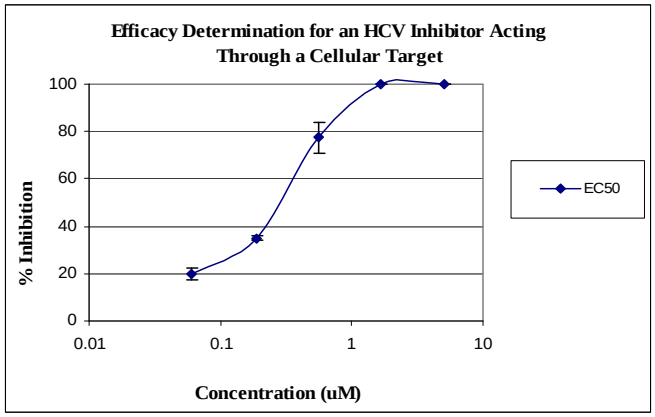

Figure 5. EC_{50} determination in APV24 infection assay in which the Test Article is an HCV inhibitor that acts through a cellular target. The assayed was performed as described in Figure 2. EC_{50} = 0.17 uM

2) Time-of-Addition Assay: Test articles are added at defined time points before, coincident with, and after the addition of infectious HCV virus that expresses *Renilla* luciferase. *Renilla* luciferase levels are quantified 48 hours after test article addition to determine the effect of the timing of Test Article addition on virus entry/replication

Raw data format: *Renilla* luciferase expression in Light Counts Per Second (LCPS)
Calculated data format: "Percent of Control" relative to solvent vehicle treated control
Signal-to-noise ratio: >500

HCV Inhibitor Performance:

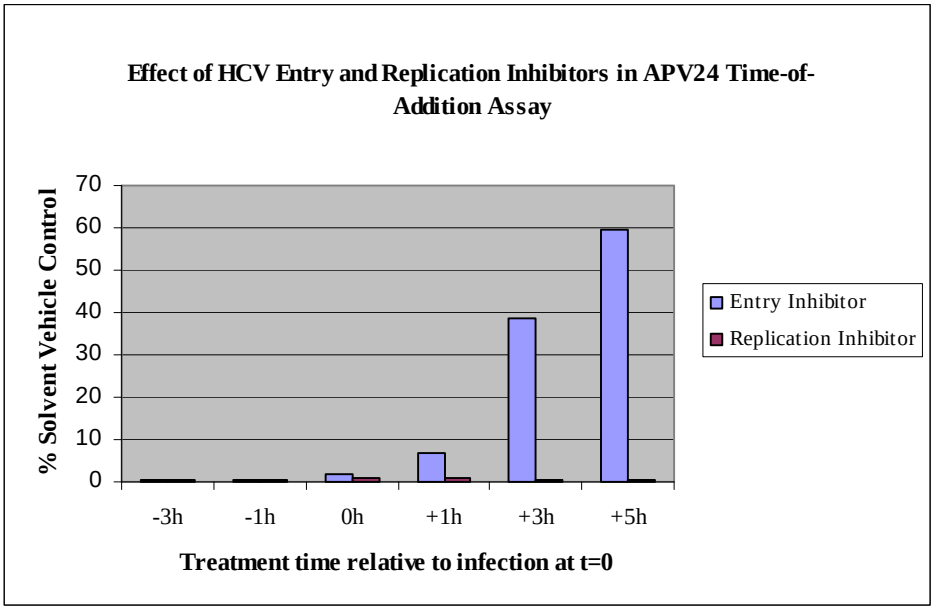

Figure 6. Time-of-addition assay comparing time-dependent efficacy of entry and replication inhibitors. Cells were seeded 24 hours prior to infection. An HCV entry or replication inhibitor was added prior to infection (-3h or -1h), coincident with infection (0h), after infection (+1h, +3h, or +5h). The assay proceeded for 48 hours, at which time Renilla luciferase activity was measured.