**R-1479**

**Cat. No.:** HY-10444  
**CAS No.:** 478182-28-4  
**Molecular Formula:** C₉H₁₂N₆O₅  
**Molecular Weight:** 284.23  
**Target:** HCV  
**Pathway:** Anti-infection  
**Storage:**  
- Powder: -20°C 3 years  
  4°C 2 years  
- In solvent: -80°C 6 months  
  -20°C 1 month

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**Solvent & Solubility**

**In Vitro**

DMSO: ≥ 50 mg/mL (175.91 mM)  
*"≥" means soluble, but saturation unknown.*

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Mass Concentration 1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>3.5183 mL</td>
<td>17.5914 mL</td>
<td>35.1828 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.7037 mL</td>
<td>3.5183 mL</td>
<td>7.0366 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.3518 mL</td>
<td>1.7591 mL</td>
<td>3.5183 mL</td>
</tr>
</tbody>
</table>

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**

1. Add each solvent one by one: **10% DMSO >> 90% (20% SBE-β-CD in saline)**  
   Solubility: ≥ 2.5 mg/mL (8.80 mM); Clear solution

2. Add each solvent one by one: **10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline**  
   Solubility: ≥ 2.5 mg/mL (8.80 mM); Clear solution

3. Add each solvent one by one: **10% DMSO >> 90% corn oil**  
   Solubility: ≥ 2.5 mg/mL (8.80 mM); Clear solution

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**BIOLOGICAL ACTIVITY**

**Description**

R-1479 is a specific inhibitor of HCV replication in the HCV subgenomic replicon system (IC₅₀=1.28 μM).

**IC₅₀ & Target**

IC₅₀: 1.28 μM (HCV replication)

**In Vitro**

R-1479 (R1479) inhibits HCV RNA replication with a mean IC₅₀ value of 1.28 μM when measured as dose-dependent
reduction of Renilla luciferase activity after a 72 h incubation of proliferating replicon cells. R-1479 shows no effect on cell viability or proliferation of HCV replicon or Huh-7 cells at concentrations up to 2 mM [1]. The most potent and non-cytotoxic derivative is R-1479 with an IC_{50} of 1.28 μM in the HCV replicon system. The triphosphate of R-1479 is prepared and shown to be an inhibitor of RNA synthesis mediated by NS5B (IC_{50}=320 nM), the RNA polymerase encoded by HCV. R-1479 displays good activity in the replicon assay with no measurable cytotoxic or cytostatic effect [2].

**PROTOCOL**

### Kinase Assay [1]

The membrane-associated, native HCV replicase complex is isolated from 2209-23 HCV replicon cells and a derived cell line carrying HCV replicon RNA with a S282T mutation in the NS5B coding sequence. The in vitro replicase assay contain 10 μL of cytoplasmic membrane fraction, 50 mM HEPES (pH 7.5), 10 mM KCl, 10 mM dithiothreitol, 5 mM MgCl_2, 20 μg/mL actinomycin D, 1 mM ATP, 1 mM GTP, 1 mM UTP, 30 μCi of [α-³³P]CTP (3000 Ci/mmol, 10 mCi/mL), 1 unit/μL SUPERase•In, 10 mM creatine phosphate, and 200 μg/mL creatine phosphokinase in a final volume of 25 μL.

Inhibition by nucleotide analogs is determined [1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

### Cell Assay [1]

The effect of R-1479 on the incorporation of tritiated thymidine into cellular DNA is measured using the [³H]thymidine incorporation scintillation proximity assay system. MTT and WST-1 assay systems are used to measure cell viability. The ATP bioluminescence assay kit HSII is used to measure intracellular ATP levels [1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**CUSTOMER VALIDATION**

- J Infect Dis. 2016 Sep 1;214(5):707-11.

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**REFERENCES**
