

Influenza Antiviral Drug Development Polymerase Inhibition Assay

Introduction

Only two classes of drugs are currently approved for the treatment of influenza: M2 ion channel blockers (adamantanes) and neuraminidase inhibitors. Adamantanes block influenza A virus entry into the host cell but have no activity against influenza B viruses. They are associated with serious side effects, and resistant viruses have emerged. Neuraminidase inhibitors block the release of virions from the host cell. Although they exhibit activity against both influenza A and B viruses, they can cause side effects and be rendered inactive by resistance. Thus, a significant need exists for novel influenza virus inhibitors, especially those directed against alternative viral targets.

Influenza virus RNA-dependent RNA polymerase catalyzes RNA transcription and viral replication. The polymerase is essential for these functions and is highly conserved, thus making the polymerase an attractive target for the development of drugs with broad efficacy against all influenza virus strains.

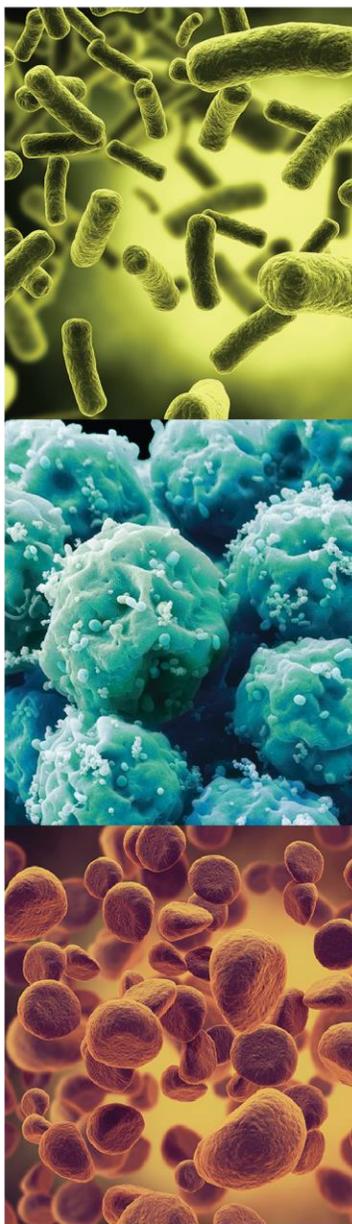
ImQuest BioSciences has optimized a rapid and sensitive influenza polymerase activity assay for the identification of novel inhibitors. Information obtained from this assay can help confirm the mechanism of action of your antiviral compound and aid in the analysis and selection of lead compound(s) for future inhibitor development.

Methodology

The assay was optimized following methods adapted from published research (1, 2). Detergent-disrupted purified virions served as the source of influenza ribonucleoprotein complex (RNP) containing the RNA-dependent-RNA-polymerase and template RNA. RNPs represent the minimal transcriptional and replicative machinery of the virus.

The RNP solution was incubated with reaction buffer containing ribonucleotides, [α - 32 P] GTP, and the dinucleotide primer ApG. Reactions, prepared in triplicate, were incubated at 30°C for 1 hour, followed immediately by the transfer of the reactions onto glass-fiber filter plates and subsequent precipitation of nucleic acids with 10% trichloroacetic acid (TCA). The filters were then washed with 5% TCA followed by 95% ethanol and air dried. Radioactivity was measured in a Microbeta liquid scintillation counter.

Negative control reactions were prepared by omitting RNP complexes whereas positive control reactions for polymerase inhibitors contained the specific inhibitor 2'-deoxy-2'-fluoroguanosine 5'-triphosphate (2DFGTP) (2).



ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products. We specialize in the development of drugs, vaccines and biologic products for the treatment and prevention of infectious disease, cancer and inflammatory disease.

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Results

The results demonstrate that the incorporation of radioactive GTP into TCA-precipitable material increases with increasing levels of RNP solution, the source of the influenza virus polymerase in reaction mixtures (Figure 1). In negative control reactions (no RNP complex), incorporation of radioactive GTP into TCA-precipitable material was negligible.

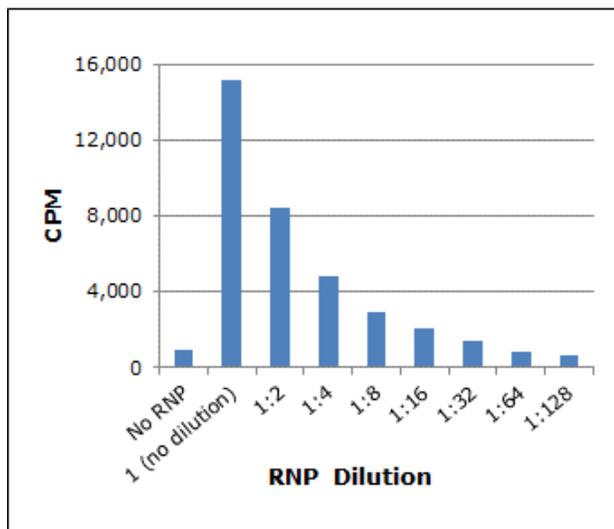


Figure 1. Incorporation of radioactive GTP into TCA-precipitable material measured as counts per minute (CPM).

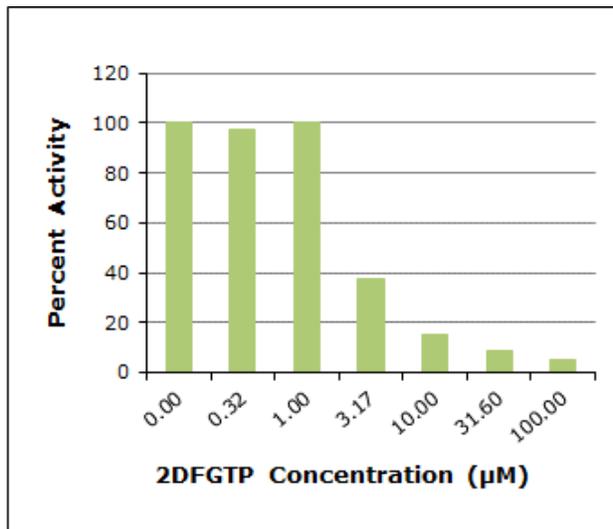


Figure 2. Inhibition of the incorporation of radioactive GTP into TCA-precipitable material by 2DFGTP.

The addition of 2DFGTP, an influenza polymerase inhibitor, decreased the incorporation of radioactive GTP into TCA-precipitable material (Figure 2). A concentration of 100 µM 2DFGTP led to a reduction of 97% of polymerase activity as compared to the polymerase alone (0 µM) control.

Using the rapid and sensitive influenza polymerase assay described above, ImQuest BioSciences can help establish the mechanism of action of your antiviral compound and assist you in the selection of lead compound(s) for future inhibitor development.

References:

1. Furuta Y., *et al.* Mechanism of Action of T-705 against Influenza Virus. *Antimicrobial Agents and Chemotherapy*. 2005; 49(3):981-986
2. Tisdale M., *et al.* Inhibition of Influenza Virus Transcription by 2'-Deoxy-2'-Fluoroguanosine. *Antimicrobial Agents and Chemotherapy*. 2005 (11): 2454-2458

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