Targeting OAS Enzymes with the Transcreener® 2-5A OAS Assay

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Overview

Oligoadenylate synthetases, including OAS1, OAS2, OAS3, are pattern recognition receptors that comprise an important component of the innate immune response against invading pathogens. Binding of dsRNA activates OAS-proteins to produce linear 2'-5' oligoadenylates (2-5A), which in turn activate endoribonuclease L (RNase L), resulting in degradation of viral RNA.

OAS enzymes and the closely related dsDNA-sensing PRR, cyclic GAMP synthase, are emerging as potential therapeutic targets for autoimmune diseases and cancer. To enable screening for small molecule modulators of OAS enzymes and for selectivity profiling of cGAS modulators, we developed a robust, HTS-compatible assay method for measuring OAS activity by using a coupling enzyme to convert 2-5A to AMP, which is then detected with the Transcreener AMP^2/GMP^2 Assay, a highly validated HTS assay method.

Because the 2'-5' oligoadenylates produced by OAS enzymes are not available commercially, we used an iterative empirical approach to optimize OAS-1 and coupling enzyme concentrations to ensure that all the product formed was converted to AMP. As expected, the reaction was dependent on the presence of dsRNA and ATP. We were able to obtain robust assay signals (>100mP) with less than 10 nM OAS1 and approximately 100 nM OAS2; OAS1 yielded a Z' value of 0.87. The assay was validated in a pilot screen of 1,280 pharmacologically active molecules, which identified several inhibitors, at least one of which demonstrated dose-dependency; suramin was also found to inhibit OAS1 with an IC_{50} of 162 nM. The Transcreener 2-5A OAS Assay will provide a robust tool for discovery of OAS inhibitors or selectivity profiling of compounds targeting related enzymes.

Transcreener 2-5A OAS Assay: Measure OAS Enzyme Activity with a Mix-and-Read FP Readout

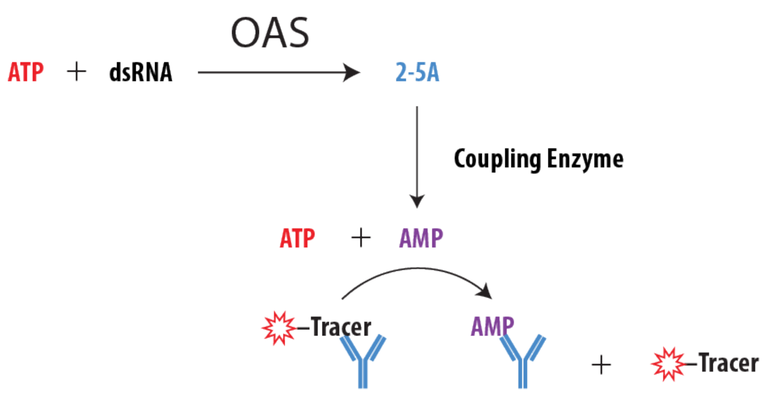

Figure 1. Schematic Overview of the Transcreener OAS FP Assay. The Transcreener 2-5A Detection Mixture contains a coupling enzyme that generates AMP and ATP from 2-5A, and an AMP AlexaFluor® 633 tracer bound to an AMP antibody. AMP produced by the coupling enzyme displaces the tracer, which rotates freely, causing a decrease in FP. The coupling enzyme regenerates ATP, preventing OAS substrate depletion.

Robust HTS-Ready Assay Procedure

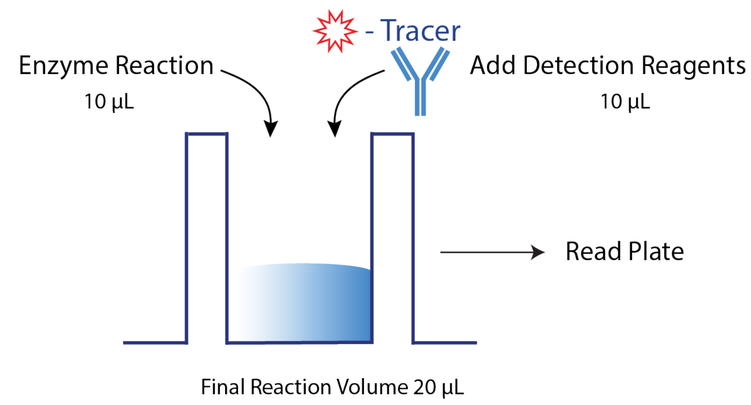

Figure 2. Schematic Overview of the Transcreener OAS FP Assay. The Transcreener OAS Assay is in a, HTS-read, mix-and-read format. Run your enzyme reaction, add detection reagents, and read the plates. 10 µL enzyme reaction followed by 10 µL detection reagents were used here (384-well format). The assay is compatible with 96, 384, and 1536-well formats.

Assay Development

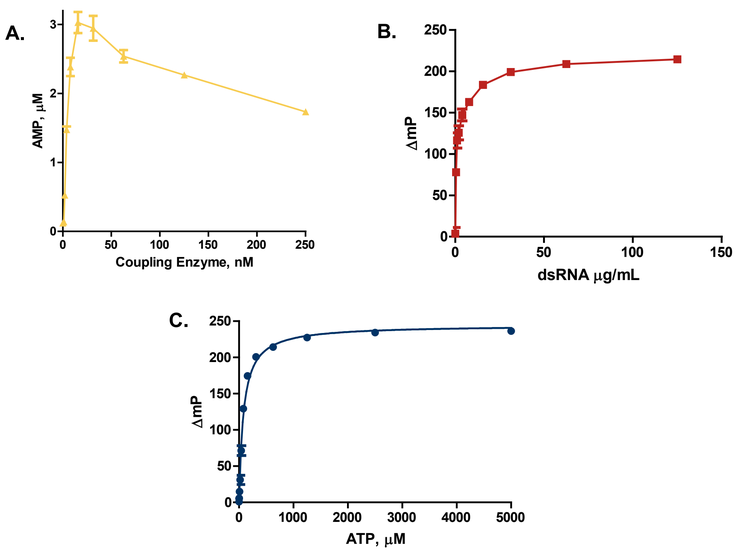

Figure 3. A. Titration of coupling enzyme in the presence of 2 nM OAS1, 1 mM ATP, and 10 µg/mL dsRNA with 32 µg/mL AMP^2/GMP^2 antibody and 2 nM tracer. **B., C.** Titration of ATP (B) and dsRNA in the presence of 2 nM OAS1, 62.5 nM coupling enzyme and Trancreener AMP^2/GMP^2 detection reagents under similar conditions. Reactions were run in continuous mode and plates were read at 60 min. Initial experiments demonstrated that neither dsRNA, ATP and coupling enzyme had no effect on the Transcreener AMP^2/GMP^2 detection reagents (data not shown).

Detection of OAS1 and OAS2

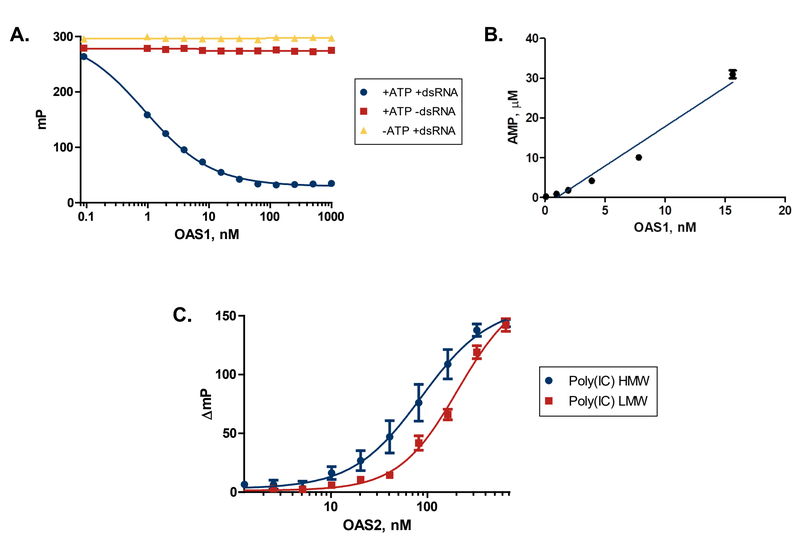

Figure 4. OAS1 Enzyme Titration. A. OAS1 was titrated in the presence and absence of acceptor substrate, 1 mM ATP and 20 µg/mL dsRNA. Buffer used was 250 mM Tris, 100 mM $MgCl_2$, 0.1% Brij-35. **B.** Data was converted to AMP formed using a standard curve, indicating linearity with enzyme concentration. Typically, a range of enzyme concentration between EC_{50} (3.37 nM) and EC_{80} (16.53 nM) is used for screening and dose response measurements. **C.** OAS2 was titrated in the presence of 20 µg/mL dsRNA, illustrating the differences in OAS response based on dsRNA size.

Z' Determination

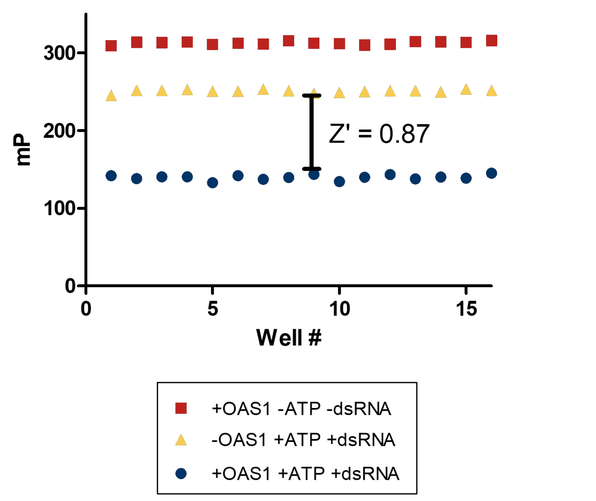

Figure 5. Assay Robustness for HTS Screening. Z' measurement using optimized OAS1 assay conditions (n=16). Z' of 0.87 demonstrates a robust assay method amenable to HTS.

Pilot Screen of 1280 Small Molecules

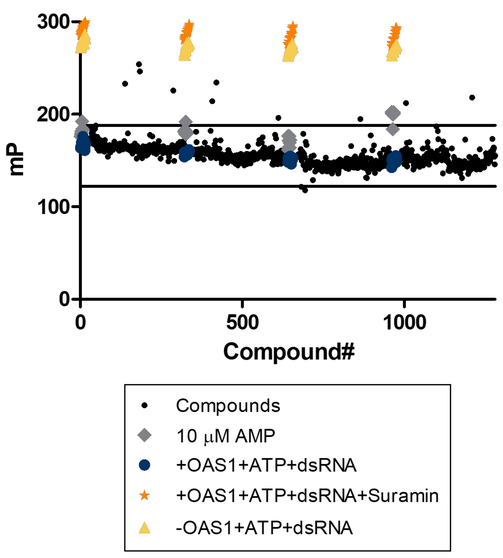

Figure 6. Pilot Screen of the Tocriscreen 2.0 Library with OAS1. 1280 compounds were screened. A total of 11 compounds were identified, with activity levels 3 standard deviations from the mean. Of these, 9 were potential inhibitors, while 2 were activators. The Z' value was 0.66.

Inhibitor Dose Response

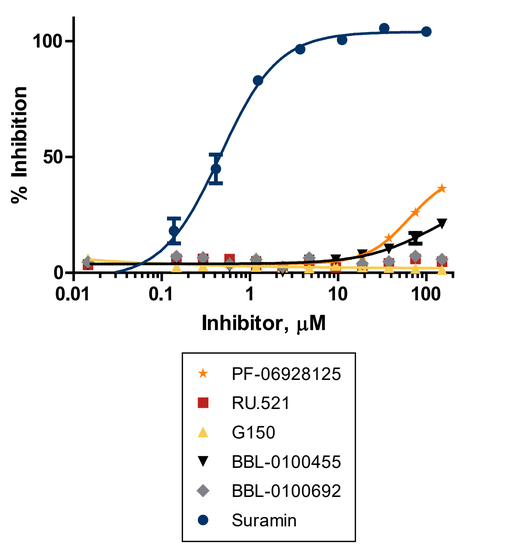

Figure 7. Inhibitor Dose Response Curve Selected hits from the pilot screen were tested in dose response mode (12 concentrations, n=2); 60-minute reaction. Plates were read in continuous mode. Dose response for Suramin, IC_{50} = 162 nM.

Conclusions

- **Coupling 2-5A conversion to AMP with the Transcreener AMP^2/GMP^2 Assay provides a robust HTS method for measuring OAS enzyme activity.**
- **The assay is highly sensitive, enabling the use of low concentrations of substrate and enzyme, minimizing cost when scaling up to large screens.**
- **The assay was able to uncover 9 potential inhibitors and 2 activators of OAS1, demonstrating the ability to accelerate screening and SAR.**
- **The new method will facilitate rapid discovery of OAS modulators for programs targeting OAS enzymes or related targets such as cGAS.**

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