

Case Study: Probing p97 ATPase with the Transcreener[®] ADP² Assay

Summary

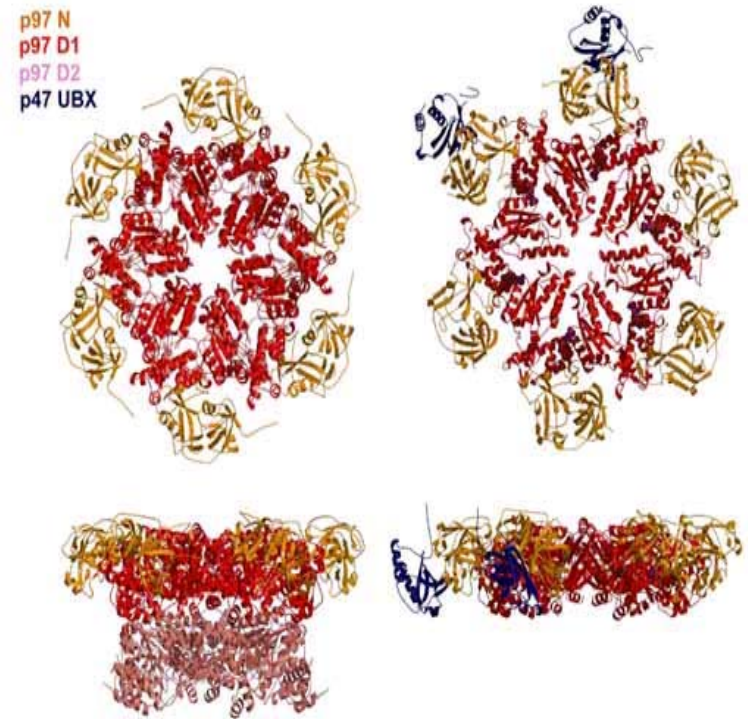
•**Background:** *p97 ATPase acts on diverse substrate proteins to partake in various cellular processes such as membrane fusion and endoplasmic reticulum-associated degradation (ERAD). The customer was expressing two different p97 enzyme preparations-Human and Mouse - but they had been unable to detect activity with the methods they were employing.*

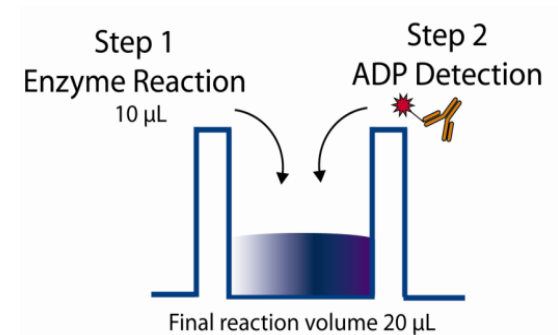
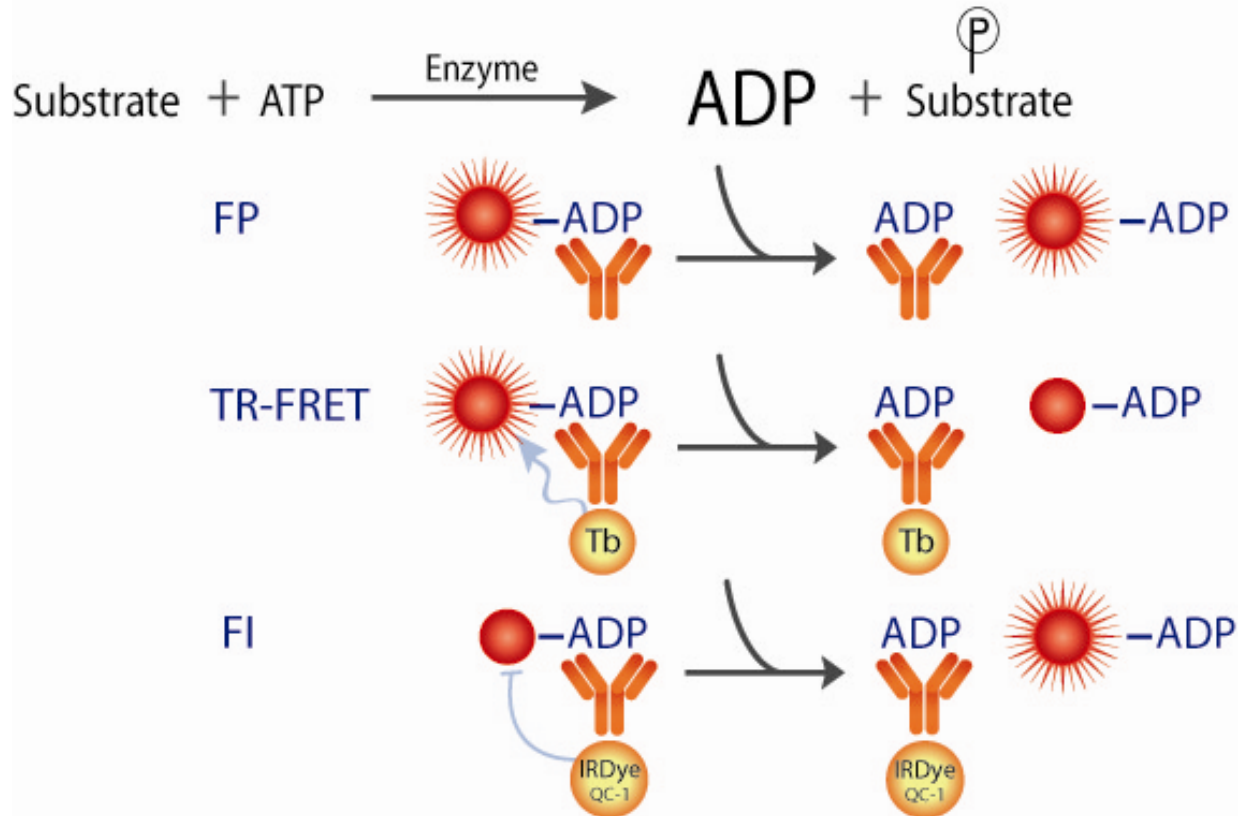
The two preparations of the enzyme were provided to BellBrook under a materials transfer agreement and an experimental plan was developed as per the customer needs.

Study Goals:

- 1) *Evaluate the activity of the two enzyme preparations and determine the optimal enzyme concentration to be used for an HTS assay.*
- 2) *Determine which of the two detection modes, FP or TR-FRET would be most suitable for this project.*

Results: *BellBrooks' scientific team optimized enzyme reaction and detection conditions that produced outstanding assay windows and Z' values for both forms of the enzyme within one week.*





•The ADP produced in the reaction is directly detected with the Transcreener ADP² monoclonal antibody using a Fluorescence Polarization, Fluorescence Intensity or TR-FRET readout.

•The assay is a simple mix-and-read format, and the signal is stable for many hours.

•A comparison of all the three detection modes can be found in this [poster](#).

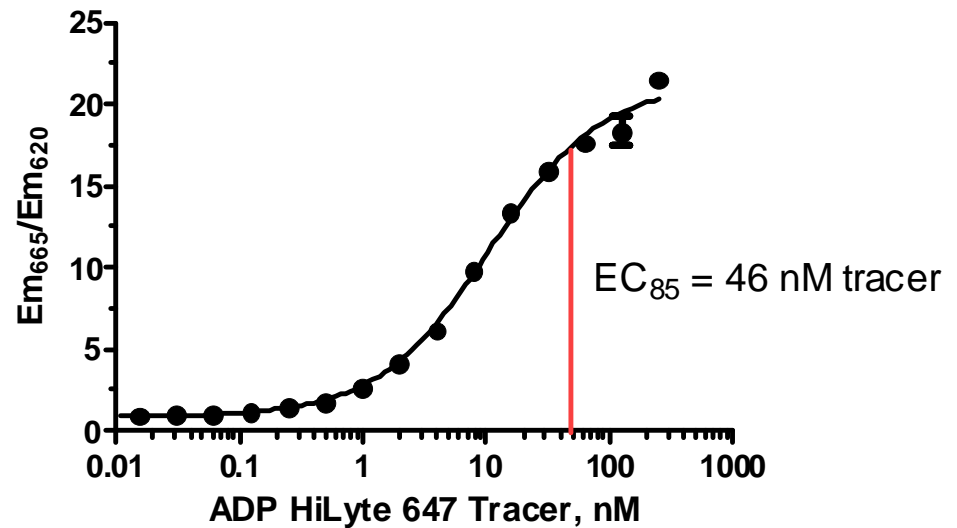
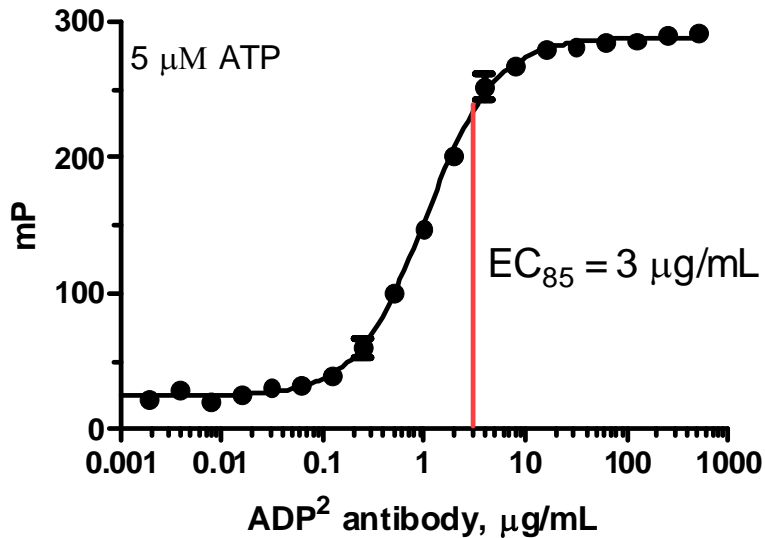
Outline of Experiments

Study goals:

- Evaluate the activity of the two p97 enzyme preparations and determine the optimal enzyme concentrations for initial velocity activity measurements.
- Suggest which of the two detection modes, FP or TR-FRET, would be best suited for this project.

- Exp 1** Assay optimization: Determine optimal reagent concentrations for the Transcreeper ADP² FP and TR-FRET Assays using p97 reaction conditions and establish standard curves for conversion of ATP to ADP.
- Exp 2** Enzyme optimization: Perform p97 titrations, both mouse and human, to determine the enzyme concentrations that yield good assay windows under initial velocity conditions (low substrate consumption) for both assay formats.
- Exp 3** Performance evaluation: Determine Z' values.

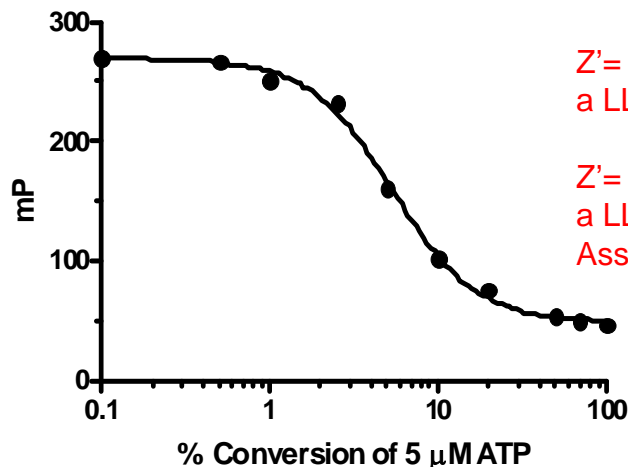
Optimizing Transcreener ADP² detection reagents for p97 reaction conditions



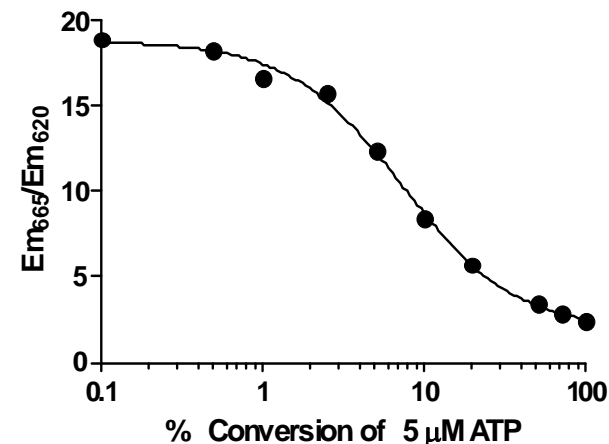
- The Transcreener ADP² antibody was titrated against a constant amount of tracer (4nM) in the presence of 5 μM ATP and reaction conditions optimal for p97 activity. The concentration of 3 $\mu\text{g/mL}$, resulting in 85% saturation (EC_{85}) yielded a good assay window for the FP assay (Fig 1).
- The Transcreener ADP HiLyte 647 tracer was titrated against a constant amount of Tb-labeled antibody (4nM) in the presence of 5 μM ATP and reaction conditions optimal for p97 activity. The concentration of 46 nM tracer, resulting in 85% saturation (EC_{85}) will yielded a good assay window for the TR-FRET assay (Fig 2).

Running 5 μM standard curves for p97 reaction conditions

5 μM ATP/ADP Standard Curve



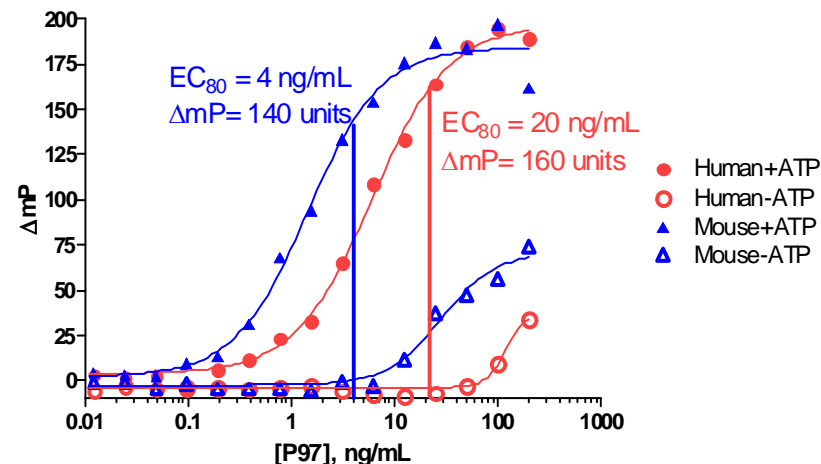
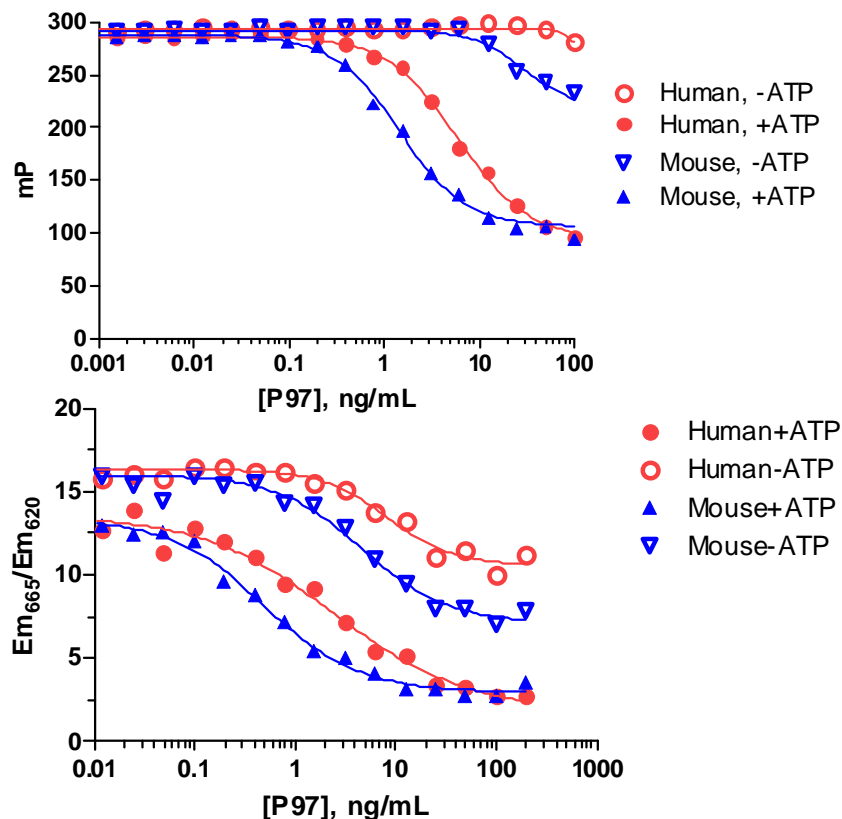
5 μM ATP/ADP Standard Curve



ADP, μM	% Conv	ΔmP	Z'
5	100	224	0.92
3.5	70	220	0.92
2.5	50	217	0.92
1	20	194	0.90
0.5	10	168	0.87
0.25	5	109	0.76
0.125	2.5	37	0.21

ADP, μM	% Conv	Z'
5	100	0.88
3.5	70	0.87
2.5	50	0.87
1	20	0.80
0.5	10	0.74
0.25	5	0.61
0.125	2.5	-0.14

- Standard curves mimicking p97 enzyme reactions (ADP is increased and ATP decreased proportionately) were established using the EC_{85} antibody concentration. Excellent Z' values were achieved at < 10% ATP conversion for both assays.



< 10% ATP was converted to ADP @ EC_{80}

- The p97 preps showed some non-specific activity even in the absence of ATP and this non-specific activity was more pronounced with the TR-FRET assay than with the FP assay.
- An excellent screening window for p97 was best achieved using the Transcreener ADP² FP Assay. The optimal (EC_{80}) concentrations for the mouse and human enzymes were 4 ng/mL and 20 ng/mL respectively.

p97 Case Study: Solutions!

- *An HTS-ready solution for screening p97 enzyme was established using the Transcreener ADP² Assay in one week.*
- An HTS-ready Transcreener ADP² FP Assay (>140 mP shift) was developed for p97 ATPase and was the better choice over the TR-FRET assay format for these particular enzyme preparations.
- Both the mouse and human p97 enzyme preparations can be used for screening, requiring only picograms of enzyme/well.
- < 10% of the ATP was consumed with both p97 assays, insuring accurate enzyme kinetics and inhibitor potencies.

1. J Biomol Screen. 2010 Mar;15(3):279-86. Epub 2010 Feb 10.
[Detection of the ATPase activity of the molecular chaperones Hsp90 and Hsp72 using the Transcreener™ ADP assay](#) Rowlands M, McAndrew C, Prodromou C, Pearl L, Kalusa A, Jones K, Workman P, Aherne W.
2. Assay Drug Dev Technol. 2011 Dec 22. [Epub ahead of print]
[High-Throughput Screening for RecA Inhibitors Using a Transcreener Adenosine 5'-O-Diphosphate Assay.](#)
Peterson EJ, Janzen WP, Kireev D, Singleton SF.
3. Assay Drug Dev Technol 2009; 7(1):56-67.
[Characterization and Optimization of a Red-Shifted Fluorescence Polarization ADP Detection Assay](#) Karen M. Kleman-Leyer, Tony A. Klink, Andrew L. Kopp, Thane A. Westermeyer, Mark D. Koeff, Brad R. Larson, Tracy J. Worzella, Cori A. Pinchard, Sebastianus A.T. van de Kar, Guido J.R. Zaman, Jorrit J. Hornberg, Robert G. Lowery.

Let BellBrook scientists help you move rapidly into your screen with confidence that it will be a success!

Available services include:

- 1) Quick enzyme activity test: We will determine the Transcreener reagent concentrations, establish a standard curve and perform an enzyme titration using user-defined reaction conditions.**
- 2) Assay optimization: We will determine the optimal enzyme concentration, buffer composition, and reaction time for maximal signal under initial velocity conditions.**
- 3) IC₅₀ determination: We will perform a 12 point dose response experiment, in triplicate, and determine the IC₅₀ with inhibitors of your choice.**
- 4) Pilot Screen: We will perform a pilot screen of 1120 small molecule drugs.**