Development of an Assay to Monitor Reductase A Activity
Brandon Hanberg, Bryce Peterson, James Twitchell, Josh Steenstra and Janice Sugiyama
InnovaBio, Utah Valley University, Ut 84058

Abstract
A human reductase A protein was cloned into pET32a (+) vector containing the following N-terminus tags: Trx-tag, His-tag, thrombin recognition sequences and S-tag. The protein was expressed in BL21 E. coli and purified via a GE HiTrap Nickel column on an AKTA FPLC. An enzyme assay was used to measure the activity of purified QR2 as described by Winger et al. 2009 [1]. Compounds, supplied by a company with vested interest in the research, were used to test inhibition potential of the reductase A protein and determine IC50.

Methods
Clone human reductase a Cloning primers were designed and used to clone reductase a into a bacterial expression vector pET32a (+), which includes N-terminus his-tag, Trx-tag and a thrombin cleavage site. Clone was sequenced verified.
Express and purity of reductase A in E. coli E. coli strain BL21 was used to express reductase a with IPTG at an OD600 of 0.600 at 30°C with shaking at 250 rpm overnight. Soluble proteins were extracted and applied to a AKTA P-1000 using a HisTrap FF crude 1 ml column from GE. An imidazole gradient was utilized to elute the protein in a more purified state. Reductase a was quantified using a DC protein assay.
Reductase a enzyme assay development Purified reductase a protein was used in a continuous spectrophotometric assay as described by Winger et al. 2009 to determine activity of the enzyme. The assay is performed in 96-well plates at 30°C with a final reaction volume of 0.2 mL. The assay is observed by the change in absorbance at 590 nm as MTT is being reduced, which is monitored by a multimode microplate reader over 15 minutes taking readings every 15 seconds.
Determine IC50 for 39 compounds Each of the compounds will be tested in triplicates at eight different concentrations. The protein and compounds will be pre-incubated and the remaining elements of the reaction will then be added and activity will be monitored the same as in the assay development. The IC50 will then be determined on 50% inhibition as compared to the control of no inhibitor.

Results
Enzyme activity assay shows the functionality of the reductase A protein after it was purified and dialyzed.

Results Continued
Western blot shows the presence of the reductase A protein in induced with IPTG.

Conclusion
The purification of the bacterial-expressed human reductase A protein was efficient and provided ample amounts of protein to use to determine the activity of the protein and then the IC50 of the 39 compounds. The activity of reductase A protein was obtained in the first experimental assay; however subsequent enzymatic assays yielded no activity.
In addition to cloning the protein with the his and trx tags, the protein was cloned without that tags and purified with an ion exchange column. It has yet to be seen if this results in a more consistently active protein.

References

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