

**Title: PTP1b Activity Assay Control Tests:  $K_m$  for Full Length and Truncated PTP1b and IC50 Determination for Sodium Vanadate ( $Na_3VO_4$ )**

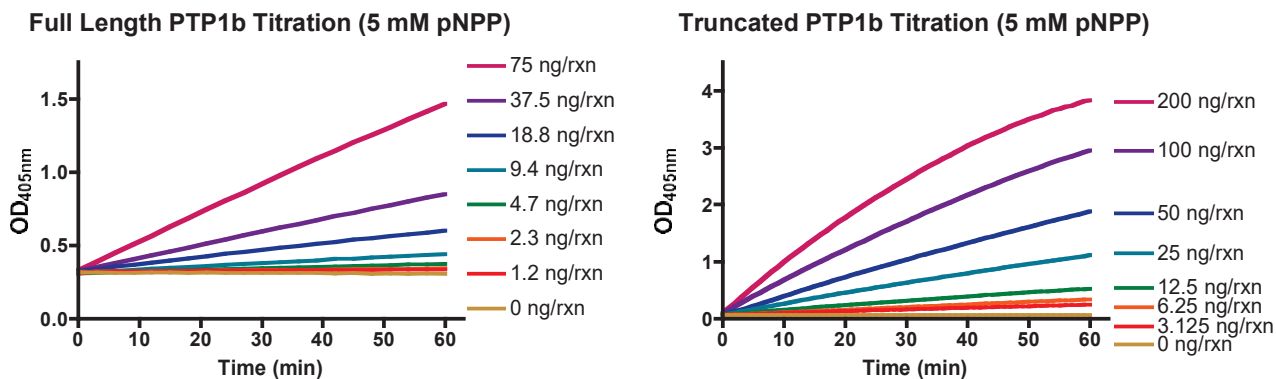
**Summary**

Before determining the IC50's for ten potential PTP1b inhibitors, we performed some initial experiments to establish appropriate assay conditions. We assessed conditions for a full length human PTP1b and a truncated human PTP1b (Glu2-Asn321). First we titrated the PTP1b's using 5 mM pNPP as the substrate. From these titrations we decided to use 50 ng/reaction for the full length PTP1b and 40 ng/rxn for the truncated PTP1b. We next titrated DMSO to determine the highest concentration that would be tolerated by each PTP1b. The  $K_m$  of pNPP for each PTP1b was then measured in the presence of the highest tolerable DMSO concentration. Finally, the IC50 for a known tyrosine phosphatase inhibitor,  $Na_3VO_4$  was measured for each PTP1b. For the full length PTP1b, we determined the maximum DMSO concentration to be 15 v% and the  $K_m$  to be  $0.7 \pm 0.04$  mM. For the truncated PTP1b, we determined the maximum DMSO concentration to be 7.5 v% and the  $K_m$  to be  $1.3 \pm 0.1$  mM. Using 0.7 mM pNPP, we observed an  $IC_{50} = 19.3 \pm 1.1$   $\mu$ M for  $Na_3VO_4$  for the full length PTP1b. Using 1 mM pNPP, we observed an  $IC_{50} = 54.5 \pm 1.1$   $\mu$ M for  $Na_3VO_4$  for the truncated PTP1b.

**Results**

**PTP1b Titrations**

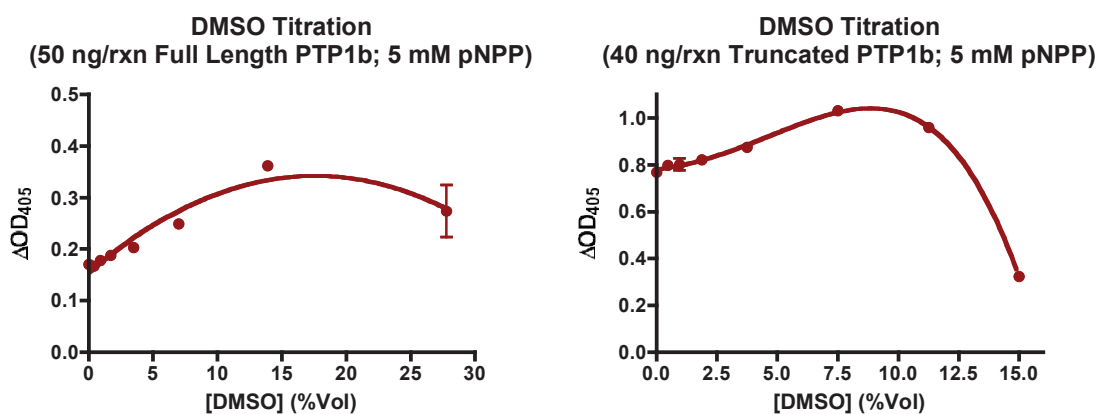
In order to determine an appropriate amount of PTP1b to use for the IC50 determinations, we titrated each PTP1b. The full length PTP1b was titrated from 75 ng/rxn and the truncated PTP1b was titrated from 200 ng/rxn. Both titrations were performed using 5 mM pNPP as the substrate. The full length PTP1b titration was performed in a 384 well plate to maximize the absorbance signal. (Due to high variation between replicates, we decided to switch to 96 well plates for the IC 50 analysis and all of the truncated PTP1b analyses.)



From these results we opted to use 50 ng/rxn for the full length PTP1b and 40 ng/rxn for the truncated PTP1b. At these concentrations the reaction would remain linear for at least 1 hr and the  $\Delta OD$  should be significant ( $>0.2$ ) even at the pNPP  $K_m$ .

## DMSO Titration

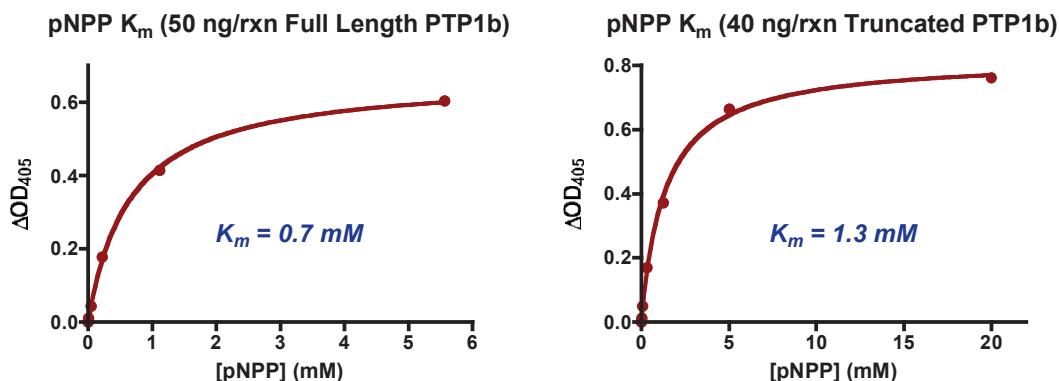
In order to determine tolerance for DMSO of each of the PTP1b's, we ran DMSO titrations for each PTP1b (from 0–27 v% for the full length PTP1b and from 0–15 v% for the truncated PTP1b). For these titrations we used 50 ng per reaction for the full length PTP1b and 40 ng truncated PTP1b.



The reactions were initiated by adding 5 mM pNPP and were allowed to proceed for 60 min. We then computed the  $\Delta OD$  ( $OD_{60min} - OD_{0min}$ ), which is proportional to the rate of reaction, for each DMSO concentration. The  $\Delta OD$ 's were then plotted versus DMSO concentration. From these results we discovered that DMSO actually enhanced the activity of each PTP1b up to a certain concentration. For the full length PTP1b, we decided to move forward with 15 v% DMSO and for the truncated PTP1b, we decided on 7.5 v% DMSO.

## $K_m$ of pNPP

In order to determine the  $K_m$  of pNPP for the PTP1b's, we titrated pNPP from 0–20 mM with either 50 ng full length PTP1b or 40 ng truncated PTP1b and computed  $\Delta OD$  ( $OD_{60min} - OD_{0min}$ ), which is proportional to the rate of reaction, for each pNPP concentration. The  $\Delta OD$ 's were then plotted versus pNPP and the  $K_m$  was computed using Prism 4 (GraphPad Software Inc.). The full length PTP1b  $K_m$  determination was performed in the presence of 15 v% DMSO and truncated PTP1b  $K_m$  determination was performed in the presence of 7.5 v% DMSO. We found the  $K_m = 0.7 \pm 0.04$  mM for the full length PTP1b and the  $K_m = 1.3 \pm 0.1$  mM for the truncated PTP1b.



### Sodium Vanadate ( $\text{Na}_3\text{VO}_4$ ) $\text{IC}_{50}$ Determination

The  $\text{IC}_{50}$  determination required two steps: 1) 30 min pre-incubation of 25  $\mu\text{L}$  PTP1b (50 ng full length PTP1b or 40 ng truncated PTP1b) with 15  $\mu\text{L}$   $\text{Na}_3\text{VO}_4$  (in 100% DMSO for full length PTP1b or 50 v% DMSO for truncated PTP1b), and 2) phosphatase reaction with 60  $\mu\text{L}$  pNPP (0.7 mM final [pNPP] for full length PTP1b and 1 mM final [pNPP] for truncated PTP1b) for 60 min. We titrated  $\text{Na}_3\text{VO}_4$  from 0-1000  $\mu\text{M}$  and computed the  $\Delta\text{OD}$  for each  $\text{Na}_3\text{VO}_4$  concentration. The  $\Delta\text{OD}$ 's were then plotted versus the Log [ $\text{Na}_3\text{VO}_4$ ] and the  $\text{IC}_{50}$  was computed using Prism 4 (GraphPad Software Inc.). We observed an  $\text{IC}_{50} = 19.3 \pm 1.1 \mu\text{M}$  for the full length PTP1b and an  $\text{IC}_{50} = 54.5 \pm 1.1 \mu\text{M}$  for the truncated PTP1b.

