P_i release assay – Mo-Sb-tartrate-VitC-Na_3citrate method

Rxn buffer:

25 mM Tris-HCl **pH 7.0** (*or 7.5 or 8.0*)
150 mM NaCl
10 mM $MgCl_2$
2 mM EGTA

Standard curve: 0 + 0.1 + 0.2 + 0.3 + 0.4 mM P_i from **KH_2PO_4 stocks (20-100 mM)**

Basic assay setup (rxn volume is 20 ul)

	Final conc.	**BLN**	**CFTR**	**CFTR boiled**
Rxn buffer	-	11 ul	11 ul	11 ul
40 mM TCEP	2 mM	1 ul	1 ul	1 ul
20 mM DDM	1 mM	1 ul	1 ul	1 ul
20 mM Na-ATP (pH7.5)	2 mM	2 ul	2 ul	2 ul
CFTR pur.	~ 80 nM	0	5 ul	5 ul *
Buffer C + desthiobiotin	-	5 ul	0	0

* 10 min @ 90 °C then cooled to RT

Rxn is started w/ addition of Na-ATP to tubes, rxns are placed to 37 °C (cabinet) for (typically) 1 hr

Coloring solutions:

A – 0.5 mM Ammonium molybdate tetrahydrate (Sigma 09878-25G): 10 mg in 6 ml H_2O + 4 ml 1 N H_2SO_4* (in 30 ml falcon), dissolve, then add 160 ul 4 mM K-Antimony(III)-tartrate hydrate (Sigma 244791-100G) (stock in H_2O), mix

B – 10 mM L-ascorbic acid (Sigma A5960-25G): 28 mg in 6 ml H_2O (in 7 ml bijoux)

Mix **A** and **B** coloring solutions in a 30 ml falcon and protect it from light. Use one coloring solution per day, but prepare fresh on each experimental day.

* 1 N H_2SO_4: 2.8 ml 96 % H_2SO_4/ 100 ml H_2O

Ceasing / Coloring of reaction:

20 ul P_i rxn (or standard) + 180 ul coloring mix (*Total volume 200 ul*)

Incubate at RT for 10 min

Add 4 ul 400 mM Na_3-citrate dihydrate (Sigma S1804-500G) (stock in H_2O)

Incubate at RT for 20 min

Detection

IMPLEN: 10 mm slit (narrow quartz cuvette), wavescan (400-950 nm), analyze Abs @ **715 nm**.

Connect to PC and export xls file. Fit standard with linear trendline (w/ 0 intercept) in excel and calculate concentration of releazed Pi from slope.