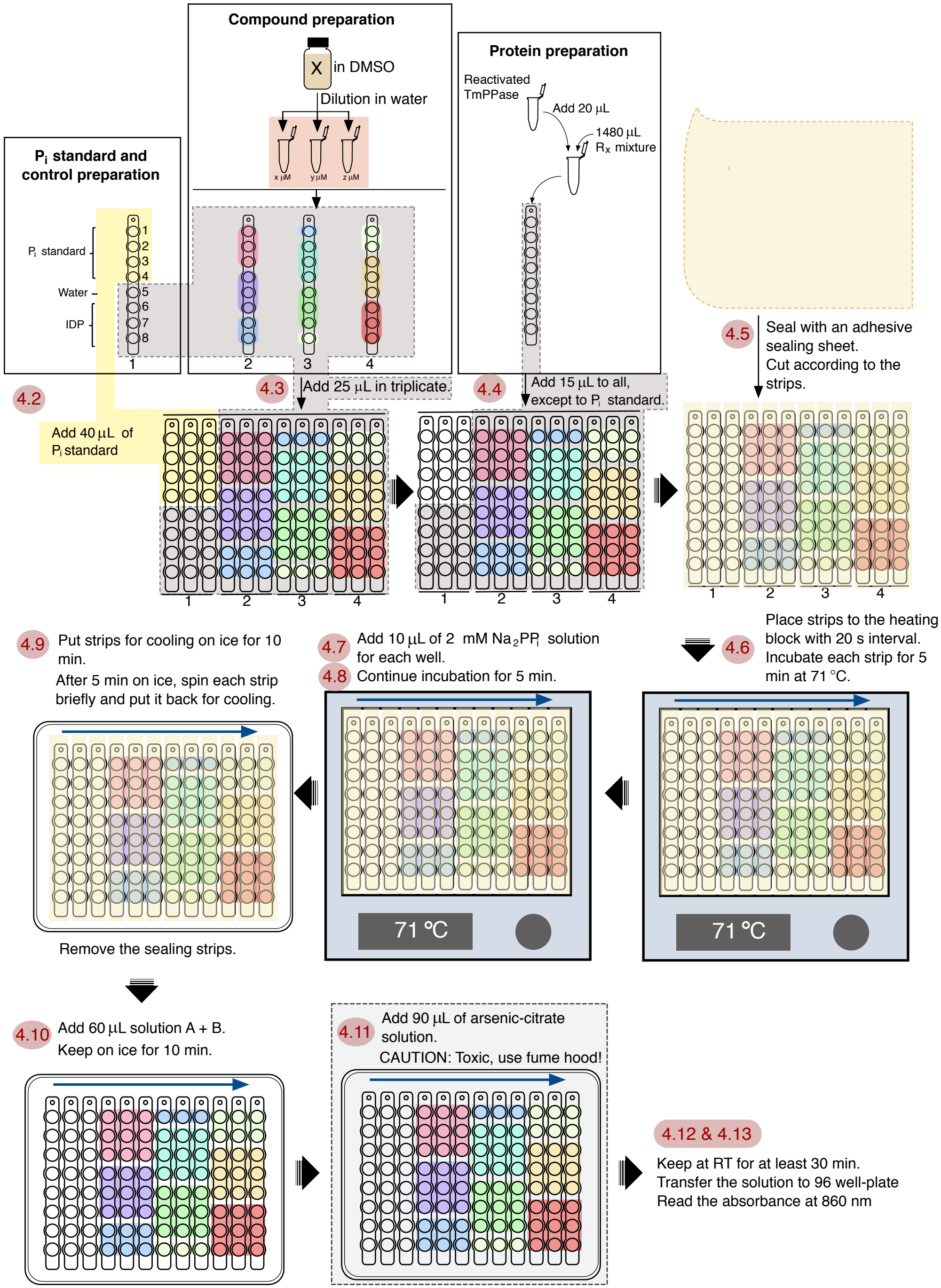
Figure 1


Compound preparation
X in DMSO
Dilution in water
x µM
y µM
z µM
Protein preparation
Reactivated TmPPase
Add 20 µL
1480 µL
Rx mixture
Pi standard and control preparation
Pi standard
Water
IDP
1
2
3
4
5
6
7
8
4.2
Add 40 µL of Pi standard
4.3
Add 25 µL in triplicate.
4.4
Add 15 µL to all, except to Pi standard.
4.5
Seal with an adhesive sealing sheet.
Cut according to the strips.
4.6
Place strips to the heating block with 20 s interval.
Incubate each strip for 5 min at 71 °C.
71 °C
4.7
Add 10 µL of 2 mM Na2PPi solution for each well.
4.8
Continue incubation for 5 min.
4.9
Put strips for cooling on ice for 10 min.
After 5 min on ice, spin each strip briefly and put it back for cooling.
Remove the sealing strips.
4.10
Add 60 µL solution A + B.
Keep on ice for 10 min.
4.11
Add 90 µL of arsenic-citrate solution.
CAUTION: Toxic, use fume hood!
4.12 & 4.13
Keep at RT for at least 30 min.
Transfer the solution to 96 well-plate
Read the absorbance at 860 nm