

Before starting...

Suspend Dowex-1 (Bio Rad AG-X2) **Be careful! The smell is awful!**

- 1) Pick 1.3g add 2mM Tris, pH 8 of 10 ml.
(Calculation: Swelled 0.65g Dowex will be 1ml. This final swelled volume is counted as 1 volume.)
- 2) Repeat washing 5-10 times (Use low speed centrifuge machine, 3740rpm, 4 degree)
Since the resin is very big and heavy, it's impossible to such the buffer completely, but don't worry. Suck the supernatant as much as possible and repeat to wash it.)
- 3) Add 1 volume of buffer after the last centrifuge.
- 4) Store at 4 degree and works for 6-7 months.

How to remove free nucleotide

- 1) Shake Dowex very well and pick 50ul before the resin precipitate into a Spin-X tube (Coster #8160).
- 2) Spin 30 sec (speed max =14K rpm) at Room Temperature for removing supernatant.
- 3) Suck and throw away the bottom solution (= storage buffer of Dowex).
*Exchanging to a new tube (only the bottom part) may be easier and cleaner.
- 4) Add 200 ul G-actin (G-buff must contain less than 0.2mM ATP) into the top side of the SpinX and mix actin with Dowex (Don't dry Dowex, add actin right after the centrifuge).
- 5) Mix gently by using the end to end mixer (occasional inversion is required) at 4 degree at 5 min.
- 6) Spin 30 sec. at 4 degree (speed max) and pick the bottom solution (actin is in there).
- 7) Incubate [x] uM actin with [x 50] uM e-ATP on ice for 2 hrs.