

Myosin ATPase Assay with [γ - ^{32}P]ATP

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Stock solutions

Isobutanol:benzene (or isobutanol:toluene), 1:1 in a glass bottle.

Sulfuric acid, 10 N: 30 ml concentrated H_2SO_4 and 70 ml water.

Silicotungstic acid (Fisher A289-100), 6% in water.

Stop solution: 2 parts 10 N sulfuric acid, 5 parts silicotungstic acid.

Ammonium molybdate (molybdic acid, ammonium salt, tetrahydrate; Sigma M-0878), 10% solution in water (filter; check for precipitate if >1 week old).

ATP stock, with 1-2 μl 10 mCi/ml [γ - ^{32}P]ATP (e.g., Redivue) per ml of stock (final $\sim 40,000$ cpm/2 μl).

Assay buffer: final concentrations of 15 mM HEPES pH 7.5, 1 mM MgCl_2 , 0.1 mM EGTA, 50 mM KCl (minimal Na^+).

ATPase assay

1. Mix together in siliconized 1.7-ml microfuge tubes: assay buffer, ATP stock, water, and other ingredients for all samples (final **10 μl** including enzyme). Include three blank tubes (all components except enzyme).

Triplicates are favored as the assay is somewhat fussy and outlier samples often arise. When you are experienced you can do duplicates with the expectation that the data will not be as reliable – nevertheless, I always prefer triplicates when I do the assay.

Note that myosin I β activity is sensitive to the salt concentration so [NaCl] and [KCl] must be carefully matched in all samples. Consider the salt and buffer contributions of the protein preps, particularly the actin prep.

2. Warm tubes to assay temperature. The rat myosin I β is assayed at 37°C; the frog enzyme at 30°C (and it has a very short half-life).
3. For experimental samples, start reaction by adding enzyme; vortex. Spin down the fluid with a 1-2 second spin in a nanofuge. Start a new tube every 30 seconds (inexperienced ATPasers should start a tube every 1 minute).

For actin-activated samples, premix the actin and myosin together on ice and add a single aliquot of the mixture. The actin is very viscous, so you will have to pay attention to the method of delivery to be sure that it is both accurate and precise.

4. Stop the reaction (every 30 seconds) by adding 67 μl of stop solution (I use a P200) and vortex for a second or so.

Vortexing here probably isn't necessary as the stop solution mixes well with the smaller volume of the assay sample.

5. Next, squirt in 250 μl of isobutanol:benzene with a Repeater. Use a 50 μl tip, with the Repeater set on **5**. It is critical that the volume of isobutanol:benzene added be consistent, so make sure that

there are no bubbles in the tip and make sure to refill the repeater before it runs out (I refill it after five shots).

6. Add 25 μl ammonium molybdate solution (I use Gilson Distriman) and vortex again until immediately before the next tube needs to be stopped.

I stop vortexing at times xx:26 and xx:56, allowing 4 seconds for putting the tube down and putting stop solution into the next tube by xx:30 or xx:00.

7. While vortexing, using your other hand, get the next aliquot of stop solution ready for the next tube.
8. When all of the samples have been extracted, spin the tubes at top speed in the microfuge at room temperature for 5 minutes to finish phase separation.

Doing this as soon as possible gives the most consistent results.

9. Using a P200, transfer 100 μl of the organic (top) phase to a scintillation vial.

It is difficult to be accurate in transferring the organic phase, but at least try to be as precise as possible so that there is internal consistency.

10. Add 2.5 ml of scintillation cocktail.
11. Also prepare three total samples: add 2 μl of the ATP stock and 250 μl of isobutanol:benzene to a scintillation vial, then add 10 ml scintillation fluid. Note that this total value will be 250% of the corrected value, as only 100/250 μl of the organic phase is taken for the samples.
12. Label vial #1 with name and lab; label all other vials with assay numbers. Count in the scintillation counter using program 5 (two-minute counts).