Gel Purification of ^{32}P-ATP

REAGENTS AND MATERIALS

(1) 24% Acrylamide (29:1 acryl:bis) solution in 1XTBE.
(2) Protein mini-gel apparatus.
(3) 10% APS
(4) TEMED
(5) 2X Loading buffer: 20% glycerol, 10 mM Tris, 1 mM EDTA
(6) Microfuge tube cap yellow dot stickers (~ few mm in diameter)
(7) Individually wrapped Kodak (ready-pack) Biomax MS film
Max sensitivity (cat # 1777853)
(8) Microspin column from Amicon-Millipore (Ultrafree, 0.22 uM)
(9) TE: 10 mM Tris, 1 mM EDTA

PROTOCOL

(1) Pour a 24%, 1X TBE acrylamide mini-gel. For a Bio-Rad minigel, 10 mls of gel mix is sufficient. Add 100 uL of 10% APS and 5 uL of TEMED to polymerize. Use the 10 well comb.

All the steps below except (8) should be carried out behind a shield

(2) Thaw the hot ATP at room temperature.

(3) Take about 2 uL of the hot stock and mix it with ~ 5 uL of loading dye and some bromophenol blue solution. This will be used later to mark the gel.

(4) Add 15 uL 2X loading buffer (without any dye) to 15 uL of hot ATP and load in a well. You can load into two or more adjacent wells if one well cannot accommodate 30 uL.

(5) In another well that is separated by at least one well from the sample, load some loading dye containing bromophenol blue.

(6) Run the gel at room temperature at 100 V till the bromophenol blue is ~ 1/4th of the way from the top of the gel.

(6) Remove the gel onto a blue pad/diaper and take off one plate. Cover it with saran wrap. On top of the saran wrap put 4-5 small dot stickers (~ few mm in diameter) surrounding the lane where ATP was loaded. Spot ~1 uL of the dye + hot ATP mix on each dot. When dry, these colored radioactive spots will be used to align the film.

(7) When the spots are dry, cover with another sheet of saran wrap. Take an individually wrapped film and expose the saran wrap covered gel for ~ 5-10 seconds. Do not remove the film wrapping. The wrapping allows exposure to radiation without light exposure so the gel can be exposed outside the dark room on a regular bench top.

(8) Take the covered film to the dark room and put through the film developer. When removing the wrapper, remember that the side of the wrapper facing the gel may be slightly hot, so dispose it off in radiation waste if necessary.

(9) Put the developed film below the gel plate. Align the film using the hot dots.

(10) After the film is aligned with the gel, remove the saranwrap and cut out the ATP band with a clean blade. The ATP should run more slowly than the free phosphate and should be the major band.

(11) Transfer the gel piece to a 1.5 mL eppendorf tube and add 200 uL of TE. Break up the gel fragment with a pipette tip. Keep at 4 ^{o}C overnight.

(12) Next day put the gel piece and supernatant through a micro-spin column. This gets rid of the gel pieces. The flow through contains the eluted hot ATP. This can be aliquoted and stored at -20 ^{o}C.