Mot1 ATPase assay

Each reaction contains:

4 ul 5 X BB
1 ul 20 mM DTT
1 ul 500 uM cold ATP
0.2 ul gamma 32P ATP (NEN NEG 502A, 10 mCi/ml, 3000 Ci/mmol)
2.0 ul 1% Brij 58
0.5 ul 0.5 ug/ul poly dG-dC

Use pencil to lightly mark Bakerflex PEI TLC plates with a line 2.5 cm from bottom edge and make light marks for origins at 2 cm intervals along the line (9 spots per plate). Set up one tube for each time point which contains 0.5 ul 20 mM EDTA and 0.5 ul 1 mM cold ATP. Remove 2 ul reaction for zero time point, mix with EDTA/ATP, quick spin and spot 2 ul on TLC plate.

Initiate reactions by addition of Mot1 to 7 nM final concentration +/- TBP (Km for TBP is 50-100 nM)

For total [ATP] in the range of 1- 50 uM, take 8 time points at 1.25 minute intervals (10 minute time course). For [ATP] greater than 50 uM, take time points at 2 minute intervals (16 minute time course). Develop TLCs in 0.6 M KH_2PO_4 (pH 3.4). (81.65 g/l; pH with HCl) Running solvent to about half height is sufficient.

Air dry TLCs, then wrap in plastic wrap and expose to film or phosphorimager. (Ten to twenty minutes exposure is sufficient for phosphorimager image.)