

Non-specific (promoter independent) RNA polymerase I activity assay

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this protocol is used to detect RNA polymerases in plant cell-free extracts or chromatography fractions due to the ability of RNA polymerases to catalyze the incorporation of radioactive nucleotide triphosphates into RNA. To assay RNA polymerases II or III, simply leave out the alpha-amanitin.

References: The protocol below is a modification of:

- 1) Roeder, R.G. (1974). JBC 249:241-248.
- 2) Schwartz et. al. (Roeder lab). 1974. JBC 249:5889-5897.

A) Solutions needed

i) 2X reaction stock mix (using alpha-labeled ^{32}P -GTP as the radioactive label):

buffer composition

100mM HEPES-KOH, pH 7.9
4mM MnCl_2 (Manganese!)
1mM UTP, (Sigma U1006)
1mM rATP, (not deoxy; Sigma A6559)
1mM rCTP (not deoxy; Sigma C8552)
0.08mM cold rGTP (Sigma G3776)
2mg/ml BSA (acetylated, Boehringer)
10ug/ml -amanitin (Sigma A2263)
50ug/ml calf thymus template DNA
100mM KCl
sterile water

recipe for 1ml

100ul 1M stock
4ul 1M stock
10ul 100mM stock
10ul 100mM stock
10ul 100mM stock
0.8ul 100mM stock
200ul 10mg/ml stock
10ul 1mg/ml stock
100ul 0.5 mg/ml stock
50ul 2M stock
505 ul

ii) 0.5 M sodium phosphate, dibasic (Na_2HPO_4)

268 grams added very slowly to 2 liters Milli Q water that is vigorously stirring with a stir bar. Let each clump of crystals dissolve before adding more, or they clump up and never dissolve. This may take an hour to make.

Procedure

- 1) dialyze all fractions to be tested into CB100 (25mM HEPES, pH7.9, 20% glycerol, 100mM KCl, 1mM DTT, 0.1mM EDTA).
- 2) determine the number of assays to be run and make the hot mix:
 - A) aliquot to a new tube 20ul of 2X mix per reaction (for 10 assays, you will need 200ul)
 - B) add 0.25ul alpha ^{32}P -GTP (1uCi/ul) per reaction (for 12 assays, add 3ul).
- 3) Pipette 20ul of dialyzed fractions to be tested into numbered microfuge tubes.
- 4) add 20ul of hot mix, vortex at low speed.
- 5) Incubate 20-30 min at room temperature.
- 6) Spot each reaction onto DEAE -81 paper squares (uniform in size for each sample) numbered with pencil and laying on parafilm or saran wrap. After each sample is spotted, immerse the filter immediately in 50ml 0.5M Na_2HPO_4 . Free UTP will wash off, but polynucleotides will be

retained. All the filters are pooled in the same beaker.

7) Wash the filters 5min with agitation on shaker platform. Pour off wash. Repeat 5min washes a total of four times.

8) Wash filters twice for 5min each with 95% ethanol.

9) wash once with as small a volume as possible (20ml ?) diethyl ether to remove the ethanol and promote rapid drying of the filters.

10) air dry filters.

11) place filters in scintillation vials, add cocktail.

12) count in scintillation counter (Julio uses: Program 2, user 4).

13) plot incorporated cpm vs. fraction number.

NOTE: Shearing the DNA template sometimes helps provide more broken ends for RNA synthesis to be initiated.