Protocol: TP30 & TP50 Prep (Nierhaus Protocol: From Spedding)

IMPORTANT: Everything should be done at 4 ºC! Use ice-cold Buffers, Rotors, etc. Use RNase free lab wares and reagents.

1. Take 300 A260 units of 30S or 50S subunits in 1 ml Buffer 3, add 0.1 ml (0.1 volume) 1 M Mg acetate and 2.2 ml (2 volumes) acetic acid.

2. Stir for 45 min at 0 ºC.

3. Centrifuge at 10, 000 g for 30 min.

4. Take the supernatant and add 16.5 ml (5 volumes) acetone and keep at –20 ºC overnight.

5. Centrifuge at 10, 000 g for 30 min.

6. Take the pellet and remove the residual acetone by placing for 30 min in a desiccator.

7. Resuspend the pellet in 1 ml Buffer 5, yielding about 300 e.u./ml, and dialyse overnight against the same buffer at 4 ºC.

8. Dialyse three times each for 45 min against 100 volumes of Buffer 4 at 4 ºC.

9. Centrifuge at 5000 g for 5 min.

10. Measure the absorption at 230 nm (1:100 dilution); store in small aliquots at –80 ºC.

TP50: 1 A230 unit = 220 µg = 10 e.u.
TP30: 1 A230 unit = 220 µg = 8 e.u.
TP70: 1 A230 unit = 220 µg = 10 e.u.

1 A260 unit of 50S =36 pmol = 1 e.u. TP50
1 A260 unit of 30S = 72 pmol = 1 e.u. TP30
1 A260 unit of 70S = 24 pmol = 1 e.u. TP70

BUFFERS FOR TP30 and TP50 PREP

Make buffers a day in advance and store them at 4 ºC. Add 2-mercaptoethanol to the buffers just before use.

Buffer 3:
20 mM NH_4^+-Hepes (pH 7.5)
4 mM $MgCl_2$
30 mM NH_4Cl
2 mM spermidine
0.2 mM spermine
5 mM 2-mercaptoethanol
0.5 mM EDTA

Buffer 4:
20 mM NH_4^+-Hepes (pH 7.5)
20 mM $MgCl_2$
400 mM NH_4Cl
6 mM 2-mercaptoethanol

Buffer 5 (same as buffer 4 plus 6 M Urea):
20 mM NH_4^+-Hepes (pH 7.5)
20 mM $MgCl_2$
400 mM NH_4Cl
6 mM 2-mercaptoethanol
6 M UREA

Also need: Acetic acid, Acetone, Dialysis tubing.