

ALWAYS work under the hood and wear safety gear.

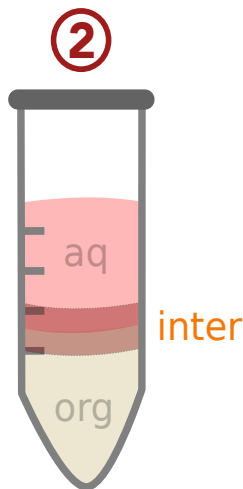
UV_{254nm} dosage must to be optimised in every case. Energies of 0.15-1.5 J/cm² have been used for mammalian cells in culture.

In a 2ml tube (safe-cap) containing 2-6x10⁶ cells in 0.6 ml PBS

Add:
0.2 ml neutral phenol
0.2 ml toluol
0.2 ml 1,3-bromo-chloro-propane (BCP)

Mix 1min, RT in Thermomixer 2.000 r.p.m.
or Vortex max. speed.
Centrifuge 20.000xg, 3 min, 4°C.

Carefully transfer 0.4-0.5 ml of the resulting Aq to a new 2 ml tube containing 0.3 ml solution D, mix pipeting.



Add:
0.6 ml neutral phenol
0.2 ml BCP

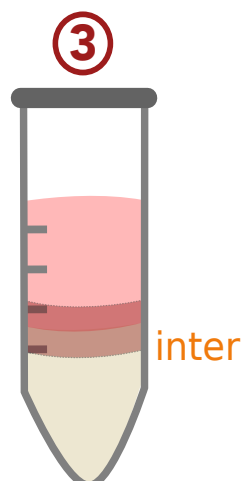
Mix 1min, RT in Thermomixer 2.000 r.p.m.
or Vortex max. speed.

Centrifuge 20.000xg, 3 min, 4°C.

Solution D:

5.85 M guanidine isothiocyanate
31.1 M sodium citrate.
25.6 mM N-lauryosyl-sarcosine
1% 2-mercaptoethanol

With a syringe and blunt needle (21G) remove 3/4 of the upper aq and 3/4 of the lower org. Keep the interphase in the same tube



Add:
0.2 ml ethanol
0.4 ml H₂O, mix briefly
0.4 ml neutral phenol
0.2 ml BCP

Mix 1min, RT in Thermomixer 2.000 r.p.m.
or Vortex max. speed.

Centrifuge 20.000xg, 3 min, 4°C.

With a new syringe remove 3/4 of the upper aq and 3/4 of the lower org.

Transfer the interphase to a 5mL tube and add 9 vol. of ethanol. Incubate 30 min at 20°C (or overnight).

Centrifuge at 20.000 xg, 30 min, 4°C. Dissolve the pellet in 30-50 µl H₂O or Laemmli buffer