

Methanol Fix a la Esther

Protocol

1. Drop 1ml of culture into 10ml of ice cold 80% MeOH
2. Let stand for 60' at room temperature.
3. Add 200µl of 16% paraformaldehyde (this step fixes the nucleoids.)
4. Let stand for 5' at room temperature.
5. Spin down cells at relatively low speed (3500RPM in a Sorvall T6000D centrifuge) and resuspend gently (no vortexing...finger flicking is best) in 1ml of ice cold 80% MeOH. The cells will tend to clump together-don't worry about it. Cells should keep for a week or so at 4°C.

Solutions:

80% MeOH -20°C

16% Paraformaldehyde

This protocol is a variation of that described by:

Hiraga S, Ichinose C, Niki H, Yamazoe M (1998) Cell cycle-dependent duplication and bidirectional migration of SeqA-associated DNA-protein complexes in E. coli.
Mol Cell 1(3):381-7