[^{3}H]Thymidine Incorporation

Solutions and Reagents Needed:

Krebs-Ringer bicarbonate buffer

Dulbecco's Modified Eagle's Medium (DMEM)

[^{3}H] Thymidine

Procedure:

1. Seed 80,000 cells in a 22.6 mm well and grown for 24 hours in standard medium.

2. Remove the growth medium and replaced with DMEM containing the desired additives (10% FCS, 0.1% BSA, 0.1% BSA plus EGF etc.) Incubate for 8 hr.

3. Pulse label the cells with 5 μCi/ml [^{3}H]thymidine for 1 hour between the 7th and 8th hour of the above treatment.

4. At the end of the pulse labeling, transfer cells to ice and wash twice with Krebs-Ringer bicarbonate buffer (KRH) . Precipitate cells by addition of KRH containing 5% trichloroacetic acid.

5. Wash the monolayers once with KRH containing 5% trichloroacetic acid, twice with KRH and once with methanol.

6. Dissolve the monolayers by treatment with 0.2 N NaOH at 37° C.

7. Remove samples to scintillation vials and count for [^{3}H].