Incorporation of Sterols into Cells Using Cholesterol/Cyclodextrin Complexes

Reagents Needed:

Dulbecco's Modified Eagle's Medium (DME) (no serum added)
Bovine serum albumen (100 mg/ml)
2.5 M Hepes, pH 7.2
Cholesterol/Cyclodextrin Complexes (6.8 mM cholesterol in 70 mM cyclodextrin)
Phosphate-buffered saline (PBS)

Procedure:

This procedure is for use with 60 mm diameter dishes of cells. Scale up or down according to the cell number to be used (ie by surface area of dish)

1. Add 25 mM HEPES and 1 mg/ml BSA to DME. Warm to 37° in the tissue culture incubator. Use 3 ml DME per 60 mm dish.
2. Add cholesterol/cyclodextrin complexes to DME/BSA/HEPES to desired concentration (usually 0.2 mM cholesterol = 30 μl complexes/ml medium). Add the complexes right before the mixture will be added to the cells since the cholesterol tends to precipitate out upon standing.
3. Wash cells once in warm PBS. Add 3 ml DME/HEPES/BSA containing cholesterol/cyclodextrin complexes.
4. Incubate 30 min at 37° C, swirling every 10 min to redistribute the medium
5. Remove medium and treat as desired.

Cells may be stimulated with growth factors and hormones during the last few minutes of treatment and then immediately processed for analysis.