Pulse and chase labeling with [35S]-Methionine

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This protocol is prepared for 12 well-plates. Adjust volume accordingly for different plates. It is recommended to plate cells in Fibronectin 20 μg/ml and use them at a confluence of 70-80%

Solutions

- Pulse-labeling medium: Met- Cys-free DMEM (Gibco Cat # 21013). It can also contain 10% dialyzed serum
- Chase medium: Met-Cys-free medium containing 7.5 g/L of Methionine. It is recommended to use Met at a concentration of 15 mg/L

Labeling of cells

- Thaw [35S]-Methionine (Perkin-Elmer, 5mCi. Cat # NEG009L005MC) at RT and prepare a 0.1-0.2 mCi/ml working solution in pre-warmed (37°C) pulse-labeling medium
- Wash cells twice with 1 ml of pulse-labeling medium
- Add 0.5 ml pulse-labeling medium and incubate 15 min at 37°C (to deplete intracellular pool of Meth)
- *Pulse*: Add 0.35 ml of [35S]-Meth working solution. Incubate 30 min at 37°C
- *Chase*: Add 0.75 ml of chase medium to the [35S]-Meth solution and remove
- Wash once with 1 ml of pre-warmed chase medium
- Add complete medium (DMEM + 10% FBS). Treatments, such as Wnt3a or Lactacystin, can also be added at this point
- Extract cells and determine [35S]-Meth incorporation by TCA precipitation

TCA precipitation

- Pre-block pieces of Whatman 3 MM paper with 0.1% Methionine. Dry
- Spot 10 μl of sample into pre-blocked paper, dry and place in cold TCA 10% (Fisher Scientific Cat # A322-500) for 20 min
- Transfer filters to a boiling solution of 5% TCA and keep for 15 min
- Wash filters once with 5% TCA
- Wash filters once with 95% Ethanol and air dry
- Determine radioactivity by scintillation counting and correct by protein content