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Cell Synchronization by Double Thymidine Block

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[Abstract] Cell synchronization is widely used in studying mechanisms involves in regulation of cell cycle progression. Through synchronization, cells at distinct cell cycle stage could be obtained. Thymidine is a DNA synthesis inhibitor that can arrest cell at G1/S boundary, prior to DNA replication. Here, we present the protocol to synchronize cells at G1/S boundary by using double thymidine block. After release into normal medium, cell population at distinct cell cycle phase could be collected at different time points.
Keywords: Cell synchronization, Cell cycle, Thymidine, DNA synthesis, DNA replication

[Background] Cell cycle and cell division lie at the heart of cell biology. To build multicellular organism, cell duplication is necessary to generate specialized cells, which can execute particular function. The normal cell cycle is composed of interphase (G1, S and G2 phase) and mitotic (M) phase (Rodríguez-Ubreva *et al.*, 2010; Léger *et al.*, 2016). During interphase, the genetic materials are duplicated and make everything ready for mitosis. Whereas, during mitotic phase, the duplicated chromosomes are segregated and distributed into daughter cells (Sakaue-Sawano *et al.*, 2008).

To precisely preserve genetic information, cell cycle progression must be tightly regulated. Cyclin/CDK complexes control the cell cycle progression through rapidly promoting activities at their respective stages, and are quickly inactivated when their stages are completed (Graña and Reddy, 1995).

Cell synchronization is particularly useful for investigating a cell-cycle regulated event. Using different methods, cells could be synchronized at different cell cycle stage. Treatment of nocodazole, which is an inhibitor of microtubule formation, could synchronize cells at G2/M phase (Ho *et al.*, 2001), while, hydroxyurea, a dNTP synthesis inhibitor, synchronize cells at early S phase (Koç *et al.*, 2004). As an Inhibitor of DNA synthesis (Schvartzman *et al.*, 1984), thymidine can arrest cell at G1/S boundary. Here, we describe a detail method to synchronize cells at G1/S boundary by thymidine (Chen *et al.*, 2018).

Materials and Reagents

1. 10 cm culture dish (Corning, catalog number: 430167)
2. Gloves (VWR International, catalog number: 82026)
3. Protective clothing (VWR International, catalog number: 414004-444)
4. Eyewear (VWR International, catalog number: 89187-984)

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5. Human tumor cell lines: H1299 (ATCC, catalog number: ATCC® CRL-5803™)
6. Dulbecco's Modified Eagle's Medium (DMEM) (high glucose with L-glutamine) (Corning, catalog number: 10-013-CV)
7. Phosphate-Buffered Saline (PBS) (Corning, catalog number: 21-040-CV)
8. Fetal bovine serum (FBS) (ATLANTA BIOLOGICALS, catalog number: S11150)
9. Thymidine (Sigma-Aldrich, catalog number: T9250)
10. Propidium Iodide (PI) (Thermo Fisher Scientific, catalog number: P3566)
11. Antibodies
    a. Anti-Cyclin A (Abcam, catalog number: ab38)
    b. Anti-Cyclin D (Santa Cruz Biotechnology, catalog number: sc-753)
    c. Anti-β-Actin (Santa Cruz Biotechnology, catalog number: sc-58673)
12. Tris-HCl, pH 8.0 (Thermo Fisher Scientific, catalog number: 15568025)
13. NaCl (Sigma-Aldrich, catalog number: S9888)
14. NP-40 (Abcam, catalog number: ab142227)
15. EDTA (Thermo Fisher Scientific, catalog number: 15576028)
16. β-Mercaptoethanol (Sigma-Aldrich, catalog number: M6250)
17. EBC cell lysis buffer (see Recipes)
18. Electrophoresis running buffer (see Recipes)
19. Transfer buffer (see Recipes)

Equipment

1. Cell culture incubator (VWR International, model: 98000-368)
2. Flow cytometry system (BD, model: FACSLyric)
3. X-RAY Film processor (Konica Minolta Healthcare Americas, model: SRX-101A)

Procedure

1. Plate H1299 cells at 20-30% confluence in a 10 cm culture dish (2 x 10^6-3 x 10^6 cells per dish) containing 10 ml of Roswell Park Memorial Institute (RPMI) 1640 Medium supplemented with 10% Fetal Bovine Serum (FBS).
2. Incubate cells at 37 °C overnight.
3. Add thymidine to a final concentration of 2 mM.
4. Culture cells in a tissue culture incubator at 37 °C for 18 h.
5. Remove thymidine by washing cells through addition of 10 ml pre-warmed 1x PBS and discard PBS.
6. Add 10 ml of pre-warmed fresh medium and incubate for 9 h in a tissue culture incubator at 37 °C.
7. Add second round of thymidine to a final concentration of 2 mM.

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8. Culture cells at the tissue culture incubator for another 18 h at 37 °C.
9. Cells are now in G1/S boundary.
10. Release cells by washing with pre-warmed 1x PBS and incubating cells in pre-warmed fresh media. Cells are collected at 0, 2, 6, 8, 10, 12, 14, 24 h for analysis of cell cycle by DNA staining using PI, or analysis of protein by Western blot using cyclin A, cyclin D and β-Actin antibodies (Figure 1).

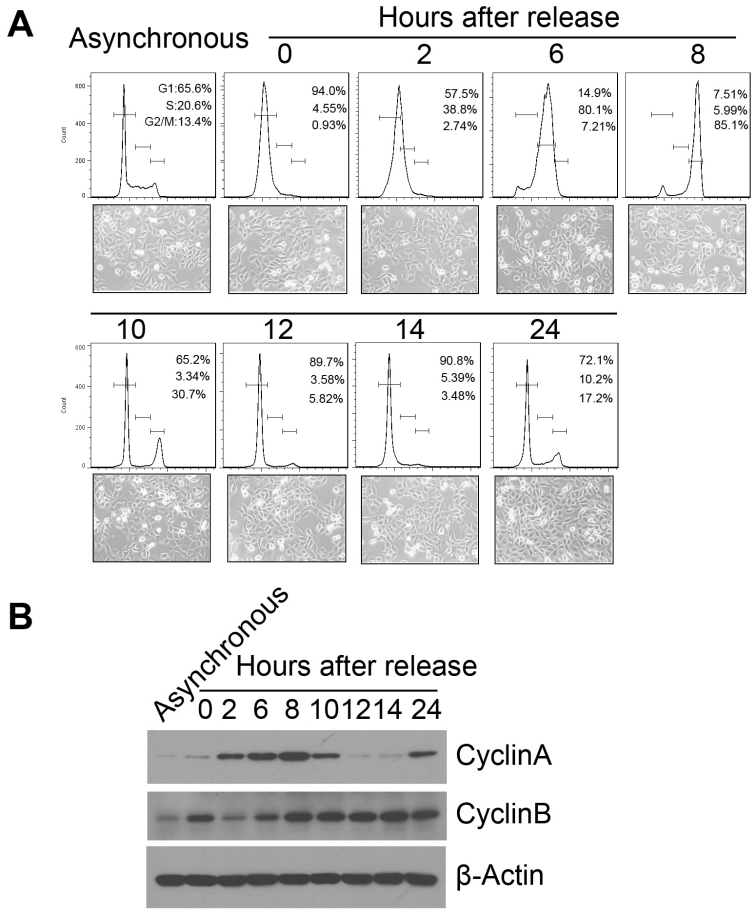

Figure 1. G1/S phase synchronized H1299 cells enter into normal cell cycle progression after release into fresh medium. A. Cell cycle profiles at indicated time points after release following double thymidine block. B. Expression levels of Cyclin A, Cyclin B and β-actin in cells at indicated time points after release.

Data analysis

Cell cycle was analyzed by flow cytometry with Flowjo software (Figure 1A). Cyclin A, Cyclin B and β-actin were detected by Western blotting (Figure 1B). Data are the representative of three independent experiments.

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Notes

1. Dissolve thymidine in PBS and make 100 mM stock solution.
2. The time points for distinct cell cycle phase are dependent on the cell cycle progression time of different cell lines.
3. Propidium Iodide (PI) is a mutagen. Gloves, protective clothing, and eyewear should be worn.

Recipes

1. EBC cell lysis buffer
   50 mM Tris-HCl pH 7.6-8.0
   120 mM NaCl
   0.5% NP-40
   1 mM EDTA
   1 mM Na_3VO_4
   50 mM NaF
   1 mM β-Mercaptoethanol
2. Electrophoresis running buffer
   25 mM Tris-HCl pH 8.3
   192 mM glycine
   0.1% SDS
3. Transfer buffer
   25 mM Tris-HCl pH 8.3
   192 mM glycine
   10% methanol
4. Cell culture medium
   Roswell Park Memorial Institute (RPMI) 1640 Medium
   10% Fetal Bovine Serum (FBS)

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Competing interests

The authors have declared that no conflict of interest exists.

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