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Cell Cycle Syncrhonization using a Double Thymidine Block

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Reagents:

Standard Cell Culture Media (example: RPMI1640 + 10% FCS)
100 mM Thymidine blocking solution
Sterile PBS or serum free media

Method:

1) Grow cells in culture in standard media to approximately 40% confluency.
2) Add 20ul of Thymidine blocking solution for each 1ml of culture media.
3) Incubate culture for exactly 19 hours.
4) Remove the media and wash culture 3x with sterile PBS.
5) Add fresh cell culture media (containing serum) and incubate for 9 hours.
6) Again add 20ul of Thymidine blocking solution for each 1ml of culture media.
7) Incubate for an additional 16 hours.
8) Remove the media and wash culture 3x with sterile PBS.
9) Add fresh culture media.

Notes:

- After the final addition of culture media the cells are synchronized in G1 and are ready to be released into cycle over the next 15-20 hours.
- Cells will remain relatively synchronous for 1-2 cell divisions.

Reference:

Bostock, C.J., D.M. Prescott and J.B. Kirkpatrick. An evaluation of the double thymidine block for synchronizing mammalian cells at the G1-S border. Experimental Cell Research 68(1): 163-168, 1971.