Easy Double Thymide block_Ledoux et al 2013, Cell Cycle.

U2OS (but also using it for others cell lines):

-treatment at 2mM for 18h (even 19h is better) => ideally at 3pm

- washed in PBS + release in normal media for 9h => release at 9am + 2nd treatment at 6pm

- treatment at 2 mM for 14h =>wash in PBS 3x + release in normal media at 8am... harvest at times wanted: usually every hour for 10-12h but will pool 2 timing : for example, after validation with FACS, t1+t2 = G1; t3+t4 = G1/S, etc.... it decreases the variation of time point during the FACS profile

More traditional method

Cell Cycle Synchronisation_Kenneth et al. Embo J. 2010.

To arrest cells at the G1/S boundary, a double thymidine block and release experiment was performed where U2OS cells were treated for 16h with 2.5mM thymidine, washed twice in PBS, released into fresh media for 10 h, treated for a further 16h with 2.5mM thymidine, then released again into fresh media following two washes in PBS. To synchronise cells in mitosis, U2OS cells were treated for 16h with 2.5mM thymidine, washed 3x in PBS, and released into fresh media containing 100 ng/ml nocodazole (Sigma) for 18h, cells were then washed 3x in PBS and released into fresh media.