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PREPARATION OF SAMPLES FOR MYCOPLASMA TESTING

1. Culture your cells for 2 weeks without any antibiotics. Split as many times during this 2 weeks as necessary just keep one flask of cells **ALWAYS** in the antibiotic free media.
2. Collect samples when cells are 80-90% confluent, **3 or more days after last split** (e.g., weekend culture) to ensure that the titer of the mycoplasmas in the medium is at a high level.
3. Collect 100 µl of media. Boil it for 5 min at 95°C. Store at 4^0C ***** (sample is stable for approximately 1 week).
4. Wash cells with PBS.
5. Remove adherent cells by cell scraper (NO TRYPSIN) into PBS.
6. Collect an aliquote for cell count and centrifuge cells at 4^0C 1200 rpm, 5 minutes.
7. Resuspend cells in PBS, place approximately 0.5-1.0 x 10^6 cells into 1.5 mL Eppendorf.
8. Centrifuge at 4^0C, 5000 rpm for 1 minute.
9. Remove supernatant.
10. Resuspend cell pellet with 1 mL PBS and centrifuge as per step 8.
11. Remove supernatant and store cell pellet at 4^0C.*****

*** The media sample as well as the cell pellet can be also stored at -20^0 C**