

Myers Lab Cell Culture Protocol for K562

This protocol describes the method used to culture K562 cells in the Myers lab at the HudsonAlpha Institute for Biotechnology. K562 is a lymphoblastoid human erythroleukemia cell line derived from a female donor. It is a suspension cell line.

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Reagents and Materials

Culture Media

500 ml RPMI 1640 + L-Glutamine (Hyclone SH30027.LS)
56 mls FBS (10%) (Gibco SH30071.03)
5.6 mls Pen/Strep (Gibco 15140-163)
5.6 mls L-Glutamine (Gibco 25030-164)

Filter sterilize. We use 1000mL Nalgene MF75 Filter Units with a 0.2 μ m CN membrane. Media should be warmed in a 37°C water bath prior to use and stored at 4°C. It should be used within 1 month.

T25 cell culture flasks (BD Falcon,VWR 353109)
T75 cell culture flasks (BD Falcon,VWR 353136)
T150 cell culture flasks (BD Falcon,VWR 355001)
T225 cell culture flasks (BD Falcon,VWR 353138)
Tissue culture dishes-150x25mm (Nalgene,VWR 127-0020)
Formaldehyde solution, for molecular biology, 36.5% (Sigma F8775-500ML)

Thawing cells

1. Add 9-10 mls of media warmed to 37°C to a 15 ml conical tube and 5 mls each to 2, T25 flasks. Place flasks in 37°C 5% CO₂ incubator.

2. Remove one vial of cells from liquid nitrogen tank and thaw vial immediately in 37°C water bath. Keep the vial's O ring above the water surface to prevent contamination. Thaw content with slight shake until only a small amount of ice remains in vial (approx. 1 min.). Spray or submerge vial in 70% EtOH and wipe surface with clean tissue in the hood.
3. Open the vial and transfer the contents to the 15 ml conical tube containing 9-10 mls of warm media. Rinse vial 2-3 times with media to ensure the majority of cells have been removed.
4. Centrifuge the thawed cells at 1200 rpm (300g) for 5 min at room temperature. We use a JOUAN CR4i tabletop swing arm centrifuge with 4x750mL rotor capacity. Return tube to hood and pour off supernatant being careful not to disturb the pellet.
5. Re-suspend cells in 10 mls fresh media warmed to 37°C and transfer 5 mls into each of the 2, T25 flasks.
6. Check the cells under microscope to ascertain presence and general morphology (irregular spheroids).
7. Culture cells in 5% CO₂ 37°C incubator and change the media approx. every 3 days following steps 3-6 above.
8. K562 cells usually take 3 days or more to recover from freezing.

Passaging cells

1. These cells reach log phase in 5-7 days. Start counting at day 3. When the cell density reaches 0.7-0.8x10⁶ cells/ml split the culture to approximately 0.4x10⁶ cells/ml with fresh, warm media. From this point, the cells should double every 24 hours.
2. Split the cells when the density reaches approx. 0.75 million/ml.
3. Place flasks in 37°C incubator with 5% CO₂.

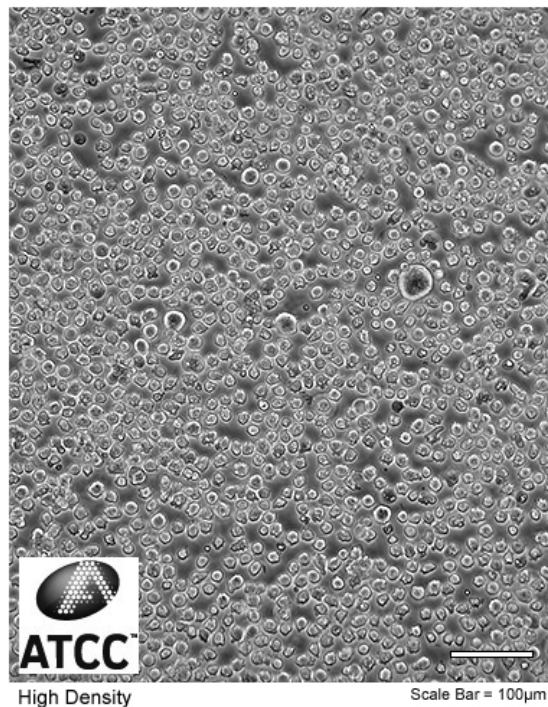
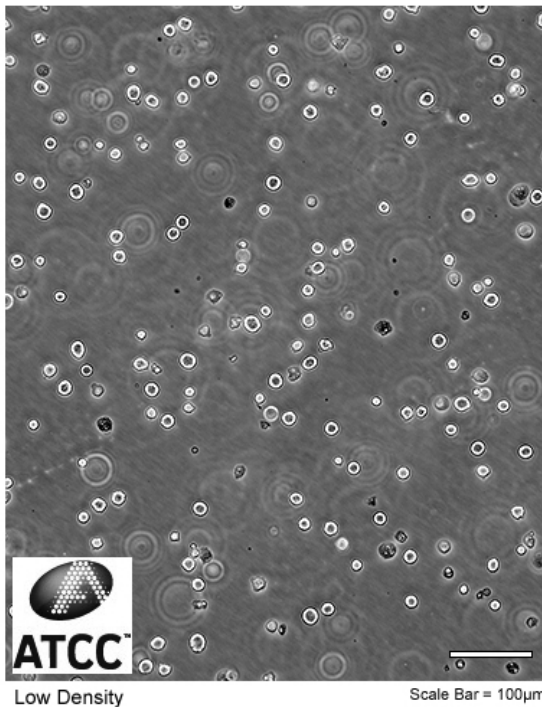
Observations

Cells should be observed every day for signs of growth to determine whether they should be fed or split. If the media appears very acidic (yellow), it may be necessary to replace the media completely. This is accomplished by adding suspended cells to 50 ml conical tubes and spinning at 1200 RPM at RT for 5 min. Pour off supernatant and re-suspend cells in warm fresh media.

<http://www.atcc.org/~media/328346C726574BE8AFA96735AAE53C15.ashx>

<http://www.atcc.org/~media/3A66015F6D96448294BD683568B8E532.ashx>

ATCC Number: **CCL-243**™
Designation: **K-562**



Harvesting cells

Formaldehyde crosslinked cells for chromatin immunoprecipitation

1. Media should be changed 1 day prior to harvesting to insure cells are growing under optimum conditions.
2. Count cells (density should not exceed 2×10^6 cells/ml) by preferred method. We utilize a standard hemocytometer.
3. Transfer cells suspended in media into 50 ml conical tubes, aliquoting in the required cell numbers. We aliquot 2.4×10^8 cells per 50 ml conical tube in no more than 45 mls of media.
4. Add formaldehyde to a final concentration of 1%, mix gently, and incubate at RT for 10 minutes.
5. Stop the reaction by adding glycine to a final concentration of 0.125 M and mix gently.
6. Pellet cells at 2000 RPM (840g) for 5 min. at 4°C.
7. Gently pour off supernatant and place tubes on ice. Add at least 10 mls PBS at 4°C and gently re-suspend cells, then centrifuge again as in step 6.
8. Gently pour off supernatant and place tubes on dry ice to snap freeze.
9. Store cells at -80°C.

Cell Pellets

1. Media should be changed 1 day prior to cell collection to insure cells are growing under optimum conditions.
2. Count cells (density should not exceed 2×10^7 cells/ml) by preferred method. We utilize a standard hemocytometer.
3. Transfer cells suspended in media into 50 ml conical tubes, aliquoting in the required cell numbers. We aliquot 6×10^7 cells per 50 ml conical tube.
4. Pellet cells at 2000 RPM (840g) for 5 min. at 4°C .
5. Gently pour off supernatant and place tubes on ice. Add at least 10 mls PBS at 4°C and gently re-suspend cells, then centrifuge again.
6. Gently pour off supernatant and place tubes on dry ice to snap freeze.
7. Store cells at -80°C .

Seed stock

1. Media should be changed 1 day prior to cell collection to insure cells are growing under optimum conditions.
2. Count cells as detailed above and aliquot desired amount of cells to be frozen (5×10^6 cells per seed stock vial) into 1 or more 50 ml conical tubes.
3. Pellet cells at 1200 RPM (300g) for 5 min. at room temperature.
4. Pour off supernatant and re-suspend pellet in FBS + 10% DMSO. (900 ul FBS + 100 ul DMSO per 5×10^6 cells)
5. Aliquot 1mL or 5×10^6 cells per cryo vial . Screw lids on loosely.
6. Place cryo vials in isopropanol jar at room temperature. Place jar in -80°C freezer overnight.
7. The following morning, remove vials from jar, fasten cryovial lids tightly, and transfer to liquid nitrogen freezer.