Table of Contents

SOP: **Propagation of K562**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

K562 may be ordered from ATCC as a frozen ampoule.

Name: K562-chronic myelogenous leukemia
ATCC #: **CCL-243**

Notes:
This cell line grows in suspension.

Materials List

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. Sodium Pyruvate (cellgro Cat# 25-000-CI)
4. T225 & T25 culture flasks
5. Graduated pipets (1, 5, 25mL)
6. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
7. Hemocytometer
8. Micropipet w/ P20 tips
9. Microscope

Growth Media for K562

RPMI 1640 with 2mM L-glutamine
Sodium Pyruvate 10mM
10% FBS
Pen-Strep (1x)

Procedure

A. Receipt of Frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T25 flask with 10ml of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) The take cell count and spin down cells, 500g for 5 minutes, then decant old media
6) Re-suspend cells in warm fresh media at a volume to yield a density of 2x10^5 cells/ml.

B. Sub-culture

1) Take cell counts every 48 to 72 hours.
2) Maintain cell density between 1x10^5 and 1x10^6 cells/ml.
3) Add fresh warm media when appropriate.
4) Record each subculture event as a passage.
5) Cells can be spun down, 500g for 5 minutes, rinsed with 1x PBS and re-suspend in a smaller volume of warm growth media when appropriate

C. Maintenance

1) Change media as cell density requires.

D. Harvest

1) Pass cells until desired cell number is achieved
2) Spin down cells and rinse with 1x PBS as described in `sub-culturing`

SOP: **Propagation of GM12878**
Date modified: **10/29/2008**
Modified by: **Jeff Goldy/M. Dorschner**

Ordering Information

GM12878 may be ordered from Coriell Cell Repositories. Proliferating cells are shipped in a T25 flask with 10-20ml of media.

To order starter cultures:

Name/Catalogue #: GM12878

Notes:
This cell line grows in suspension and should be maintained at a density between 2x10^5 cells/ml and 1x10^6cells/ml.

Materials List

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T225 culture flasks
4. Graduated pipets (1, 5, 25, 50mL)
5. Penicillin-Streptomycin Solution, 100X (Cellgro, Cat#300-002CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope
9. Freezing medium (growth medium containing 6% DMSO)

Growth Medium for GM12878

RPMI 1640 with 2mM L-glutamine
15% FBS
Pen-Strep (1X)

Procedure

A. Receipt of proliferating cells and generation of seed stocks

1) Equilibrate unopened T25 flask overnight in 37^{o}C, 5% CO_2 humidified incubator to allow cells to recover.
2) Cells should be counted the next day and split to achieve a cell density of 200,000-500,000 cells/ml.
3) Cells should be incubated in upright flasks with vented or loose caps.
4) Upon reaching the desired number, cells should be spun down, rinsed with 1X PBS, resuspended in freezing medium.
5) Cells are dispensed into cryovials (2 million per aliquot) and frozen in a -80^{o}C isopropanol bath overnight.
6) Cryovials are transferred the next day to liquid nitrogen for long term storage.

B. Sub-culture and Maintenance

1) Maintain culture at a cell density between 2x10^5 and 1x10^6 cells/ml.
2) Cells will either need to be fed again after 3-4 days or split depending on the cell density. Splitting can be performed by centrifuging cells at 500g for 5 minutes, decanting growth medium and rinsing in sterile 1X PBS. Cells should then be resuspended in fresh growth medium to achieve a density 2x10^5 and 1x10^6 cells/ml.

C. Harvest

1) Pass cells until the desired number of cells is reached.
2) Spin down and rinse cells as described above in Sub-culture and maintenance.

SOP: **Propagation of HeLa S3**
Date modified: **10/29/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

HeLa S3 may be ordered from ATCC as a frozen ampoule.

Name: HeLa S3, cervical carcinoma
ATCC #: **CCL-2.2**

Notes:
This is an adherent cell line. We use DMEM in place of ATCC recommended F-12K medium.

Materials List

1. DMEM with 2mM L-glutamine (cellgro Cat# 10-013-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T75 & T225 culture flasks
4. Graduated pipets (1, 5, 25mL)
5. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope

Growth Media for HeLa S3

DMEM with 2mM L-glutamine
10% FBS
1x Pen-Strep

Procedure

A. Receipt of Frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37C water bath
3) Transfer thawed cells to a T75 flask with 40ml of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 3 minutes (4^{o}C)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:4 to 1:8 cell split as needed
9) Record each subculture event as a passage

C. Maintenance

1) Change media the day after seeding and every 72 hours thereafter.
   Use 50ml of media per T225

D. Harvest

1) Do not use cells that have been passed more than 25 times
2) Remove cells from flasks according to protocol described above under 'sub-culturing'
3) Examine viability using trypan blue staining.

SOP: **Propagation of HepG2**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

HepG2 can be ordered from ATCC as a frozen ampoule.

Name:	HepG2, hepatocellular carcinoma
ATCC #:	HB-8065

Notes:
This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium pyruvate (Cellgro Cat# 25-000-CI)
5. Non-essential amino acids (Cellgro Cat# 25-025-CI)
6. T75 & T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for HepG2

MEM with 2mM L-glutamine
Non-essential amino acids
Sodium Pyruvate 1mM
10% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37 ^{o}C water bath
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes (4^{o}C).
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:4 to 1:6 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter.
   Use ~50mLs of medium per T225 flasks.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of primary Human Umbilical Vein Endothelial Cells (HUVECs; Lonza Biosciences)**
Date modified: **8/27/08**
Modified by: **M. Dorschner (UW)**
L. Dillon (NHGRI)

Ordering Information

Primary Human Umbilical Vein Endothelial Cells (HUVECs) may be ordered either as frozen ampules or as starter cultures. The former contain ~0.5-1 x 10^5 cells; the latter are initiated at Lonza and sent in a T225 flask containing 6-7 x 10^6 cells.

For all orders, provide (1) Reservation #; (2) Contract/quotation #; (3) Individual (Lot #); and (4) Item #s, as follows:

Reservation number: ~~RZ 495718~~ 3122124 (Updated 7/22/08)
Contract number: P101416
Individual H1: Lot #7F3239 - Male, Caucasian,
~~154 amps available~~, 0 amps available (7/22/08)

Individual H2: Lot #7F3771 - Male, African American
~~144 amps available~~, 38 amps available (7/22/08)

To order frozen ampules + media:
Name: HUVEC – Umbilical Vein Endo Cells
Item #: CC-2517 (HUVEC in EGM® - Cryopreserved ampule)
CC-3162 (EGM-2 BulletKit = CC-3156 + CC-4176)

To order starter cultures:
Name: HUVEC – Umbilical Vein Endo Cells
Item #: CC2501T225 (HUVECs in EGM® T-225 Flask)
CC-3162 (EGM-2 BulletKit = CC-3156 + CC-4176)

Notes:

The number of BulletKits purchased depends on the target number of cells to be generated. A rule of thumb is 10 BulletKits for every initial T225 flask of cells. It is strongly recommended to purchase all of the media that will be required for a complete expansion series (see below), since media supply may be erratic.

Materials List

1. Cell-type specific medium (BulletKits – Lonza Biosciences)
2. T225 culture flasks
3. Graduated pipets (1, 5, 25mL)
4. Pen-strep solution (if required; Lonza typically supplies antibiotics)
5. Hemocytometer
6. Micropipet w/ P20 tips
7. Microscope

Procedure

A. Receipt of proliferating cells

1) Equilibrate for 3-4 hours in 37^{o}C, 5% CO_2 humidified incubator.
2) Remove shipping medium. Replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 3 minutes (4^{o}C)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Count cells with hemocytometer.
9) Add warmed medium to flasks.
10) Seed flasks at **5,000 cells/cm^2**
11) Record each subculture event as a passage

C. Maintenance

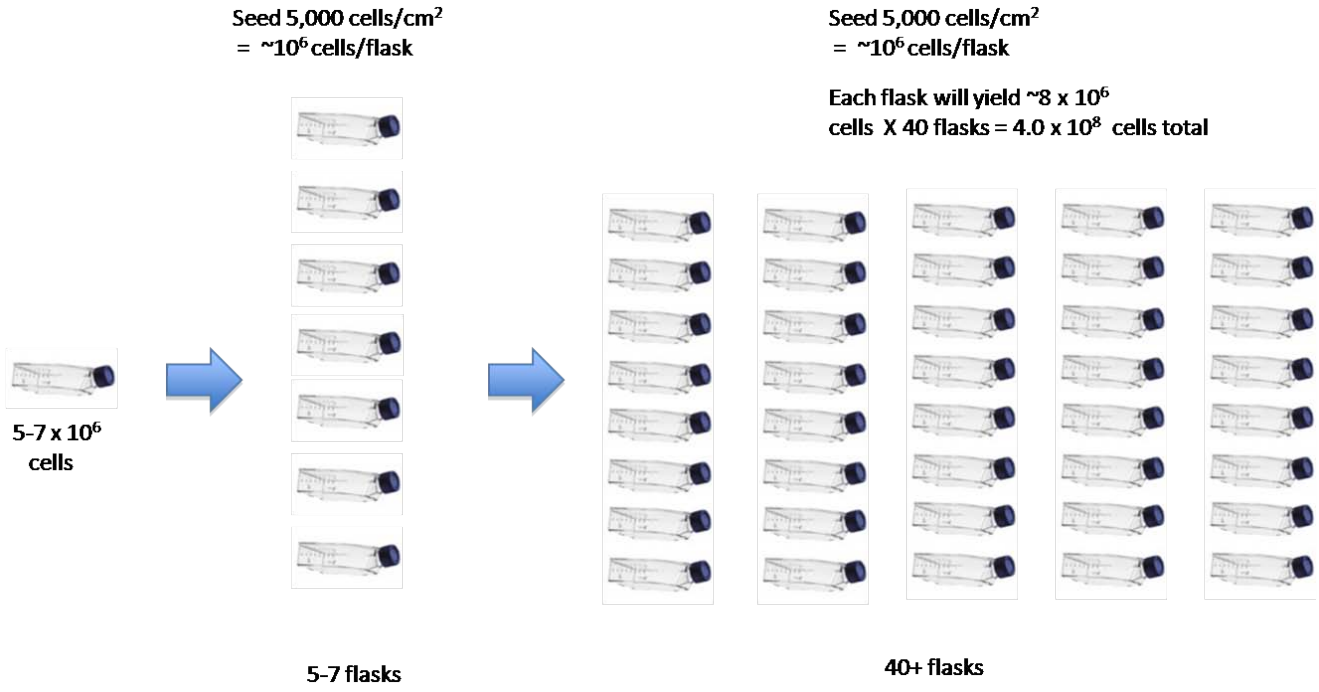
1) Change media the day after seeding and every OTHER day thereafter.
2) Increase media volume as confluency increases (volumes assume the use of
3) T225 flasks):
    a. 25 % = 1mL/5 cm^2
    b. 25-45% = 1.5mL/ 5 cm^2
    c. 45%+ = 2mL/ 5 cm^2
4) Per the above an exemplary schedule might be:
    a. day 1, plate into T225: use 50 mls of media.
    b. day 2, change media, use 50 mls of media
    c. day 4, change media, use 100 mls of media (if confluency is >50%)
    d. day 6, change media, use 100 mls of media (or harvest if ready)
    e. day 7 or 8 (harvest when cells reach 6 x 10^6 cells/flask

D. Harvest

1) Pass cells 3-4 times until the desired cell number is achieved (primary cells will senesce after 4-5 passages).
2) Remove cells from flasks according to protocol described above under 'subculturing'
3) Examine viability using trypan blue staining (SOP)

Exemplary Expansion

The diagram below illustrates an exemplary expansion of HUVECs from a Lonza starter culture:



- The initial T225 flask received from Lonza will have ~6 x 10^6 cells; this will then be split and seeded at ~3,500 cells/cm^2; each new T225 flask will therefore be seeded with ~750K cells.
- The initial flask will yield up to 7-8 daughter flasks depending on how large of an expansion is targeted.
- Once these flasks have reached the target density again, they can be split and seeded into up to 40 flasks.
- The 40 granddaughter flasks will each yield ~6 x 10^6 cells, providing a total theoretical yield of 2.5x10^8 cells.

Media requirements: Each flask will require ~50mL of medium with additional medium for feedings during the doubling/expansion process.

SOP: **Propagation of primary keratinocytes (NHEK; Lonza Biosciences)**
Date modified: **7/22/08**
Modified by: **M. Dorschner (UW)**
L. Dillon (NHGRI)

Ordering Information

Normal Human Epidermal Keratinocytes (NHEKs) may be ordered either as frozen ampules or as starter cultures. The former contain ~0.5-1 x 10^5 cells; the latter are initiated at Lonza and sent in a T225 flask containing 6-7 x 10^6 cells.

For all orders, provide (1) Reservation #; (2) Contract/quotation #; (3) Individual (Lot #); and (4) Item #s, as follows:

Reservation number:	~~RZ 495718~~ 3122124 (Updated 7/22/08)
Contract number:	P101416
Individual K1:	Lot #4F1155J - Female, African American
	~~178 amps available~~, 51 amps available (7/22/08)
Individual K2:	Lot #7F4307 - Female, Caucasian
	~~179 amps available~~; 139 amps available (7/22/08)

To order frozen ampules + media:

Name:	NHEK – Adult Keratinocytes
Item #:	CC-2501 (NHEK in KGM® - Cryopreserved ampule)
	CC-3111 (KGM BulletKit = CC-3101 + CC-4131)

To order starter cultures:

Name:	NHEK – Adult Keratinocytes
Item #:	CC2501T225 (NHEKs in KGM® T225 Flask)
	CC-3111 (KGM BulletKit = CC-3101 + CC-4131)

Notes:

The number of BulletKits purchased depends on the target number of cells to be generated. A rule of thumb is 10 BulletKits for every initial T225 flask of cells. It is strongly recommended to purchase all of the media that will be required for a complete expansion series (see below), since media supply may be erratic.

Materials List

1. Cell-type specific medium (BulletKits – Lonza Biosciences)
2. T225 culture flasks
3. Graduated pipets (1, 5, 25mL)
4. Pen-strep solution (if required; Lonza typically supplies antibiotics)
5. Hemocytometer
6. Micropipet w/ P20 tips
7. Microscope

Procedure

A. Receipt of proliferating cells

1) Equilibrate for 3-4 hours in 37^{o}C, 5% CO_2 humidified incubator.
2) Remove shipping medium. Replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 3 minutes (4^{o}C)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Count cells with hemocytometer.
9) Add warmed medium to flasks.
10) Seed flasks at **3,500 cells/cm^2**
11) Record each subculture event as a passage

C. Maintenance

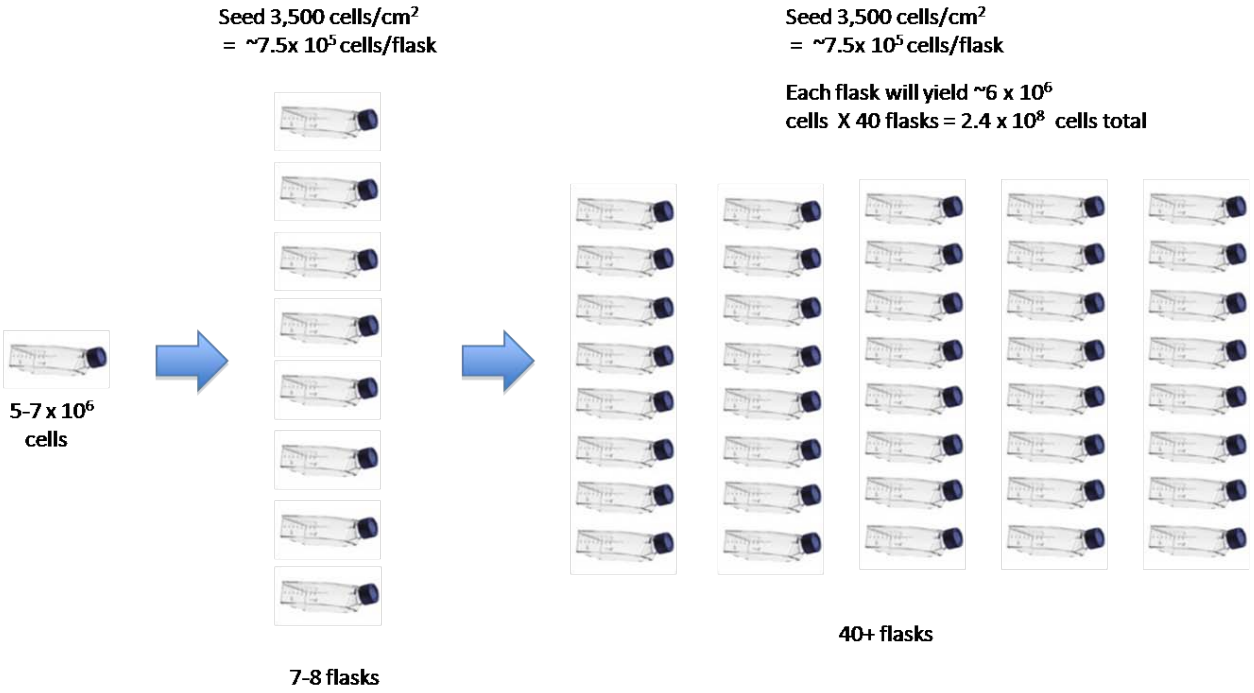
1) Change media the day after seeding and every OTHER day thereafter.
2) Increase media volume as confluency increases (volumes assume the use of
3) T225 flasks):
    a. 25 % = 1mL/5 cm2
    b. 25-45% = 1.5mL/ 5 cm2
    c. 45%+ = 2mL/ 5 cm2.
4) Per the above an exemplary schedule might be:
    a. day 1, plate into T225: use 50 mls of media.
    b. day 2, change media, use 50 mls of media
    c. day 4, change media, use 100 mls of media (if confluency is >50%)
    d. day 6, change media, use 100 mls of media (or harvest if ready)
    e. day 7 or 8 (harvest when cells reach 6 x 10^6 cells/flask

D. Harvest

1) Pass cells 3-4 times until the desired cell number is achieved (primary cells will senesce after 4-5 passages).
2) Remove cells from flasks according to protocol described above under 'subculturing'
3) Examine viability using trypan blue staining (SOP TP-7)

Exemplary Expansion

The diagram below illustrates an exemplary expansion of NHEKs from a Lonza starter culture:



- The initial T225 flask received from Lonza will have ~6 x 10^6 cells; this will then be split and seeded at ~3,500 cells/cm^2; each new T225 flask will therefore be seeded with ~750K cells.
- The initial flask will yield up to 7-8 daughter flasks depending on how large of an expansion is targeted.
- Once these flasks have reached the target density again, they can be split and seeded into up to 40 flasks.
- The 40 granddaughter flasks will each yield ~6 x 10^6 cells, providing a total theoretical yield of 2.5x10^8 cells.

Media requirements: Each flask will require ~50mL of medium with additional medium for feedings during the doubling/expansion process.

SOP: **Propagation of GM06990**
Date modified: **10/29/2008**
Modified by: **Jeff Goldy/M. Dorschner**

Ordering Information

GM06990 may be ordered from Coriell Cell Repositories. Proliferating cells are shipped in a T25 flask with 10-20ml of media.

To order starter cultures:
Name/Catalogue #: GM06990

Notes:
This cell line grows in suspension and should be maintained at a density between 2x10^5 cells/ml and 1x10^6cells/ml.

Materials List

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T225 culture flasks
4. Graduated pipets (1, 5, 25, 50mL)
5. Penicillin-Streptomycin Solution, 100X (Cellgro, Cat#300-002CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope
9. Freezing medium (growth medium containing 6% DMSO)

Growth Medium for GM06990

RPMI 1640 with 2mM L-glutamine
15% FBS
Pen-Strep (1X)

Procedure

A. Receipt of proliferating cells and generation of seed stocks

1) Equilibrate unopened T25 flask overnight in 37^{o}C, 5% CO_2 humidified incubator to allow cells to recover.
2) Cells should be counted the next day and split to achieve a cell density of 200,000-500,000 cells/ml.
3) Cells should be incubated in upright flasks with vented or loose caps.
4) Upon reaching the desired number, cells should be spun down, rinsed with 1X PBS, resuspended in freezing medium.
5) Cells are dispensed into cryovials (2 million per aliquot) and frozen in a -80^{o}C isopropanol bath overnight.
6) Cryovials are transferred the next day to liquid nitrogen for long term storage.

B. Sub-culture and Maintenance

1) Maintain culture at a cell density between 2x10^5 and 1x10^6 cells/ml.
2) Cells will either need to be fed again after 3-4 days or split depending on the cell density. Splitting can be performed by centrifuging cells at 500g for 5 minutes, decanting growth medium and rinsing in sterile 1X PBS. Cells should then be resuspended in fresh growth medium to achieve a density 2x10^5 and 1x10^6 cells/ml.

C. Harvest

1) Pass cells until the desired number of cells is reached.
2) Spin down and rinse cells as described above in Sub-culture and maintenance.

SOP: **Propagation of Sk-N-SH**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

SK-N-SH can be ordered from ATCC as a frozen ampule.

Name: Sk-N-SH, Neuroblastoma
ATCC #: HTB-11

Notes:
This is an adherent cell line. Cells are differentiated with retinoic acid (ATRA) for 48 hours prior to harvesting.

Materials List

1. RPMI 1640 with 2mM L-glutamine (Cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Pyruvate (Cellgro Cat# 25-000-CI)
4. T225 culture flasks
5. *all trans*-Retinoic acid (Sigma, Cat #R2625)
6. Graduated pipets (1, 5, 25mL)
7. Penicillin-Streptomycin Solution, 100X (Cellgro, Cat#300-002CI)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

Growth Medium for SK-N-Sh

RPMI 1640 with 2mM L-glutamine
Sodium Pyruvate 10mM
10% FBS
Pen-strep (1X)

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath
3) Transfer thawed cells to a T75 flask with 40ml of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 3 minutes ($4^{o}C$).
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:3 to 1:8 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and 1-2 times per week thereafter. Use ~50mLs of medium per T225 flask.

D. Differentiation

1) Upon reaching the desired cell number, add growth medium containing 6uM *all trans*-retinoic acid. Cells should be cultured for 48 hours in differentiation medium.

E. Harvest

1) Do not use cells that have been passed more than 8 times
2) Remove cells from flasks according to protocol described above under 'subculturing'
3) Examine viability using trypan blue staining (SOP)

SOP: **Propagation of Ovcar-3**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

Ovcar-3 can be ordered from ATCC as a frozen ampoule.

Name:	Ovcar-3, ovarian adenocarcinoma
ATCC #:	HTB-161

Notes:
This is an adherent cell line.

Materials List

1. RPMI-1640 with 2mM L-glutamine (Cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium Pyruvate (Cellgro Cat# 25-000-CI)
5. HEPES buffer (Cellgro Cat# 25-060-CI)
6. T75 & T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for Ovcar-3

RPMI-1640 with 2mM L-glutamine
HEPES Buffer 10mM
Sodium Pyruvate 1mM
20% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L
Bovine Insulin 0.01mg/ml

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes ($4^{o}C$)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:2 to 1:4 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter.
   Use ~50ml of medium per T225 flask.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Let cells grow 48 hours past confluence.
4) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of BJ-tert**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

BJ-tert can be ordered from ATCC as a frozen ampoule.

Name:	BJ-tert, skin fibroblast
ATCC #:	CRL-2522

Notes:
This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM).
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium pyruvate (Cellgro Cat# 25-000-CI)
5. Non-essential amino acids (Cellgro Cat# 25-025-CI)
6. T75 & T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for BJ-tert

MEM with 2mM L-glutamine
Non-essential amino acids
Sodium Pyruvate 1mM
10% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes (4^{o}C).
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:2 to 1:9 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter.
   Use ~50ml of medium per T225 flasks.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of Caco-2**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

Caco-2 can be ordered from ATCC as a frozen ampoule.

Name:	Caco-2, colorectal adenocarcinoma
ATCC #:	HTB-37

Notes:
This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium Pyruvate (Cellgro Cat# 25-000-CI)
5. Non-essential amino acids (Cellgro Cat# 25-025-CI)
6. T75 & T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for Caco-2

MEM with 2mM L-glutamine
Non-essential amino acids
Sodium Pyruvate 1mM
20% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches ~80% confluence (8 X 10^4 – 10^5 cells/cm^2).
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes (4^oC)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:4 to 1:8 cell split as needed. Seed at a density of 10^4cells/cm^2.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and 1- 2 times per week.
   Use ~50mLs of medium per T225 flask.

D. Differentiation

1) Upon reaching the desired cell number, cells are grown to confluence. Cells are not harvested until 2 days after confluence to ensure complete differentiation.

E. Harvest

2) Do not use cells that have been passed more than 8 times.
3) Remove cells from flasks according to protocol described above under 'subculturing'.
4) Let cells grow 48 hours past confluence.
5) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of HEK293**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

Hek293 can be ordered from ATCC as a frozen ampoule.

Name:	HEK293, embryonic kidney
ATCC #:	CRL-1573

Notes:
This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium pyruvate (Cellgro Cat# 25-000-CI)
5. Non-essential amino acids (Cellgro Cat# 25-025-CI)
6. T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for HEK293

MEM with 2mM L-glutamine
Non-essential amino acids
Sodium Pyruvate 1mM
10% FBS
Pen-strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes ($4^{o}C$)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:2 to 1:4 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter.
   Use ~50mLs of medium per T225 flask.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of MCF-7**
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

MCF-7 can be ordered from ATCC as a frozen ampoule.

Name:	MCF-7, mammary gland, adenocarcinoma
ATCC #:	HTB-22

Notes:
This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (cellgro Cat# 25-035-CI)
4. Non-essential amino acids (cellgro Cat# 25-025-CI)
5. T75 & T225 culture flasks
6. Graduated pipets (1, 5, 25mL)
7. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

Growth Medium for MCF-7

MEM with 2mM L-glutamine
Non-essential amino acids
10% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures

1) Imediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampule in 37 ^{o}C water bath.
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 5 minutes (4^{o}C).
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:3 to 1:6 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter.
   Use ~50mLs of medium per T225 flask.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of** NTERA-2
Date modified: **9/5/2008**
Modified by: **J. Goldy/M. Dorschner**

Ordering Information

NTERA-2 can be ordered from ATCC as a frozen ampoule.

Name: NTERA-2, testis-malignant pluripotent embryonal carcinoma
ATCC #: CRL-1973

Notes:
This is an adherent cell line. Cells must be scraped for subculturing and harvesting.

Materials List

1. DMEM with 2mM L-glutamine (Cellgro Cat# 10-013-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Cell Scraper (Falcon Cat# 353087)
5. T75 & T225 culture flasks
6. Graduated pipets (1, 5, 25mL)
7. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

Growth Medium for NTERA-2

DMEM with 2mM L-glutamine
10% FBS
Pen-strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Scrape cells from each flask using cell scraper.
3) Immediately remove cells each flask and pellet at 500 xg for 5 minutes ($4^{o}C$).
4) Gently re-suspend cell pellet in warm medium.
5) Perform 1:4 to 1:8 cell split as needed. New subcultures should be seeded at $\geq$ 1.5 X 10^{7} viable cells per 225 cm^{2} flask.
6) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every 2-3 days thereafter. Cultures should be maintained at high density. Use ~50mLs of medium per T225 flask.

D. Harvest

1) Do not use cells that have been passed more than 8 times.
2) Remove cells from flasks according to protocol described above under 'subculturing'.
3) Examine viability using trypan blue staining (SOP).

SOP: **Propagation of Th1**
Date modified: **11/17/2008**
Modified by: **M. Dorschner**

Source Information:

Cells are procured from primary pheresis of a single normal subject.

Notes:

Th1 T-cell subset is purified and expanded in primary culture.

Materials List

1. Naïve CD4+ T cell isolation kit (Miltenyi Biotech, Cat # 130-091-894)
2. autoMACS Separator (Miltenyi Biotech)
3. Aim V medium (Invitrogen, Cat # 087-0112DK)
4. AB serum (Lonza Bioscience, Cat # 14-490E)
5. Anti-CD3/Anti-CD28 coated beads (Dynal/Invitrogen, Cat #111-31D)
6. Human IL-2 (R&D Systems)
7. Human IL-12 (R&D Systems)
8. Anti-Human IL-4 (eBioscience)
9. Bio-Plex Human Cytokine Th1/Th2 Panel (Bio-Rad, Cat # 171-A11081)
10. T25 & T225 culture flasks
11. Graduated pipets
12. Hemocytometer
13. Phorbol 12-myristate 13-acetate (Sigma, Cat # P1585)
14. Ionomycin (Sigma, Cat # I3909)

Th1 Polarization Medium

Aim V medium
2% AB serum
Human IL-2 (50 IU/mL)
Human IL-12 (10 ng/mL)
Anti-Human IL-4 (5 ug/mL)

Procedure

A. Isolation of naïve CD4+ T cells

1) Isolate naïve CD4+ T cells by negative selection using the Naïve CD4+ T Cell Isolation Kit according to manufacturer's recommendations.

B. Stimulation and polarization of cells

1) Resuspend naïve CD4+ T cells (93% $\geq$ CD4+CD45RA+) in Aim V medium containing 2% serum.
2) Stimulate cells with anti-CD3 and anti-CD28 coated beads in polarizing medium.
3) Expand cells in culture for 7-10 days.
4) If needed, stimulate cells with PMA (2.5 ug/mL) and Ionomycin (500 uM) to divide.

C. Confirmation of Th1 polarization

1) Assay supernatant with Bio-Plex Human Cytokine Th1/Th2 panel according to manufacturer's protocol.

D. Harvest

1) Pellet cells by centrifugation and wash cells in 1X PBS.
2) Examine viability using trypan blue staining.