BIOCYTE EDUCATION AND RESEARCH FOUNDATION , SANGLI, MAHARASHTRA	
Standard operating procedures of cell line	**Revision No: 01**
	Date of Issue: 21/07/2024
Cell biology Lab. SOP NO- BiRD/CBL/1	**Effective Date:21/07/2024**

Sr no.	Cell line	Cell line Name
Cancer Cell Line		
1	MCF-7	Human; Mammary gland; Breast; Adenocarcinoma cell line
2	MDA-MB-231	Human; Adenocarcinoma; Mammary gland cell line
3	HepG2	Human; Liver Cancer cell line
4	COLO 320DM	Human Colon Cancer cell line
5	COLO 205	Human Colon Cancer cell line
6	PC-3	Human; Adenocarcinoma; Prostate cell line
7	A**549**	Human; Lung cancer cell line
8	B16-F10	Mouse; Skin; Melanoma cell line
9	HL-60	Human Leukemia cell line
10	MG-63	Human; Osteosarcoma; Bone cell line
11	SH-SY-5Y	Human: Brain; Neuroblastoma cell line
12	SKOV3	Human; Ovary Adenocarcinoma cell line
13	HCT 116	Human Colorectal adenocarcinoma Cell line
14	HT-29	Human Colorectal adenocarcinoma Cell line
15	CaCo2	Immortalized Human colorectal Cell Line
Norma	**Cell line**	
A	L929	Connective Tissue; mouse, Mus Muscular
B	Min6	Mouse Mus Musculus; Pancreatic B cells
C	RAW 264**.7**	Murine Monocyte/ Macrophage- Anti-inflammatory cell line

Human; Mammary gland; Breast; Adenocarcinoma

Growth medium: Minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1 mM Na pyruvate and supplemented with 0.01 mg/ml calf insulin, 90%; fetal calf serum, 10%

1) MCF-7

Growth conditions: Temperature- 37°C; Carbon-dioxide 5% atmosphere

Remove medium, and rinse with TPVG solution. Remove the solution and add an additional 1 to 2 ml of TPVG solution. Allow the flask to sit at room temperature until cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.

Split ratio of 1:3 is suggested

Medium change: 3 times per week

2) MDA-MB-231

Human; Adenocarcinoma; Mammary Gland
Growth Medium: Leibovitz's L-15 medium with 2 mM L-glutamine, 90%; fetal bovine serum 10%

Growth Condition: Temperature 37°C; No Carbon-dioxide 5% atmosphere

Remove growth medium from flask, Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense into new flask.

Recommended Split ratio: 1:2

Medium Change: 3 times per week

3) HepG2

Human; Liver cancer cell line

Growth Medium: Minimum essential medium (Eagle) with 2 mM L- glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM Na pyruvate, 90% fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.

Split ratio of 1:4 is recommended

Medium Change: Twice per week

4) COLO 320DM

Human; Colon cancer cell line

Growth Medium: RPMI 1640 medium adjusted to contain 1.5 g/L Na bicarbonate, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.

Split ratio of 1:5 recommended

Medium Change: 1 to 2 times per week

5) COLO 205

Human; Colon cancer cell line

Growth Medium: RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L Na bicarbonate, 4.5 g/L glucose, 10 mM HEPES and 1.0 mM Na pyruvate, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks

Split ratio of 1:3 is recommended

Medium Change: Every 2 to 3 days

6) PC-3

Human; Adenocarcinoma; Prostate

Growth medium: Ham's F12K medium with 2 mM L- glutamine adjusted to contain 1.5 g/L Na bicarbonate, 90%; fetal calf serum, 10%

Growth conditions: Temperature- 37°C; 5% Carbon-dioxide atmosphere

Remove medium, and rinse with TPVG solution. Remove the solution and add an additional 1 to 2 ml of TPVG solution. Allow the flask to sit at room temperature until cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks

Split ratio of 1:3 to 1:6 is suggested

Medium change: 2 times per week

7) A549

Human; Lung cancer cell line

Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L Na bicarbonate and 4.5 g/L glucose, 90%; fetal calf serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Remove medium and rinse with fresh medium. Remove the PBS completely and add fresh TPVG solution. Allow the flask to sit at room temperature until the cells detach. Centrifuge the cell suspension at 1000 rpm for 10 minutes, resuspend the pellet in fresh medium, aspirate and dispense into new flasks

Split ratio of 1:3 is suggested

Medium Change: Every 2 to 3 days

8) B16-F10 Mouse; Skin; Melanoma

Growth Medium: Dulbecco's modified Eagle's medium with 4 mM L- glutamine adjusted to contain 1.5 g/L Na bicarbonate and 4.5 g/L glucose, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from fiask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the call sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.

Split ratio of 1:6 is suggested

Medium Change: 3 times per week

9) HL-60

Human Leukemia

Growth Medium: RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L Na bicarbonate, 4.5 g/L glucose, and 1.0 mM Na pyruvate, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Cultures can be maintained by the addition of fresh medium or partial replacement of spent medium with fresh medium. Initiate cultures at 1 x 105 cells/ ml and maintain up to cell desity of 1 x 106 cells /ml

Medium Change: Every 2 to 3 days

10) MG-63

Human; Osteosarcoma; Bone

Growth Medium: Minimum essential medium (Eagle) with 2 mM L- glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM Na pyruvate 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.

Medium Change: 2 times per week

11) SH-SY-5Y

Split ratio of 1:4 is suggested

Human: Brain; Neuroblastoma

Growth Medium: Ham's F12K medium with 2mM L-glutamine adjusted to contain 1.5 g/L Na bicarbonate, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; 5% Carbon-dioxide atmosphere

Cells grow as a mixture of floating and adherent cells. Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.

Split ratio of 1:3 is suggested

Medium Change: Every 3-4 days

12) SKOV3

Human; Ovary Adenocarcinoma

Growth Medium: McCoy's 5A 90%; fetal bovine serum 10%

Growth Condition: Temperature 37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask, Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense into new flask.

Medium Change: Every 2 to 3 days

11) SH-SY-5Y

Recommended Split ratio: 1:3

Medium Change: Every 2 to 3 days

13) HCT-116

Human; Colon Adenocarcinoma

Growth Medium: McCoy's 5a medium 90%; fetal bovine serum 10%

Growth Condition: Temperature 37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cell come off substratum. Aspirate with fresh medium and dispense into new flask.

Recommended Split ratio: 1:3 to 1:8

Medium Change: Every 2 to 3 days

14) HT-29

Human; Colorectal adenocarcinoma

Growth Medium: Dulbecco's modified Eagle's medium with 4.5 g/L glucose adjusted to contain 1.5 g/L Na bicarbonate, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature 37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.

Split ratio of 1:3 to 1:8 is suggested

Medium Change: 3 times per week

|15) CaCo-2

Human; Adenocarcinoama

Growth Medium: Minimum essential medium (Eagle) with 2 mM L-

glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acids and 1.0 mM Na pyruvate, 80%; fetal bovine serum, 20%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from fiask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense into new flask.

Split ratio of 1:2 to 1:3 is suggested

Medium Change: 1 to 2 times per week

A) Min6

Mouse Mus Musculus; Pancreatic B cells

Growth Medium: Dulbecco's Modified Eagle's medium, 90%; fetal bovine serum 10%

Growth Condition: Temperature 37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cells come off substratum. Aspirate with fresh medium and dispense cell suspension into new flasks.
Recommended Split ratio: 1:4

Medium Change: Every 2 to 3 days

B) L929

Connective Tissue; mouse, Mus Muscular

Growth Medium: Dulbecco's Modified Eagle's medium, 90%; fetal bovine serum 10%

Growth Condition: Temperature 37°C; Carbon-dioxide 5% atmosphere

Remove growth medium from flask. Rinse with TPVG twice and remove as much TPVG as possible leaving enough so that a thin film is formed over the cell sheet. Keep flask in horizontal position for some time. Tap flask against palm of hand and cell come off substratum. Aspirate with fresh medium and dispense into new flask.

Recommended Split ratio: 1:2 to 1:8

Medium Change: Every 3rd day

C) RAW 264.7

Mouse: Abelson murine Leukemia virus induced tumor

Growth Medium: Dulbecco's modified Eagle's medium with 4 mM L- glutamine adjusted to contain 1.5 g/L Na bicarbonate and 4.5 g/L glucose, 90%; fetal bovine serum, 10%

Growth Conditions: Temperature-37°C; Carbon-dioxide 5% atmosphere

Subcultures are prepared by scraping. Remove old medium, add fresh dislodge cells and dispense into new flasks

List of Bacteria

	Gram positive strains	**Purpose**
Staphylococcus aureus	ATCC no 6538	
Bacillus subtilis	ATCC no 6633	
	Gram Negative strains	
E. coli	ATCC no 8739	
Pseudomonas aeruginosa	ATCC no 15442	
Proteus mirabilis	ATCC no 43071	
Klebsiella pneumoniae	ATCC no 13883	
	Fungal strains	
Candida albicans	ATCC no 14053	
Aspergilus niger	ATCC no 11414	
Aspergilus brasiliensis	ATCC no16404	
	Other Strain	
Proponibacterium acne	ATCC NO 11827	Antiacne activity
Malassezia furfur	ATCC no.14521-	Antidandruff activity
Mycobacterium smegmatis	ATCC no. 101-	Putative Anti Tubercular activity

Medium cange: 3 times per week