IVISbrite tumor cell culture guidelines

Revvity recommends customers thoroughly read Cell Culture Guidelines as well as product specific Technical Data sheet before working with cell lines.

As a courtesy, we have provided a second vial to be kept frozen as a backup. If there is an issue after use of the first vial, DO NOT THAW second vial; please contact at Revvity Technical Support Team at www.revvity.com

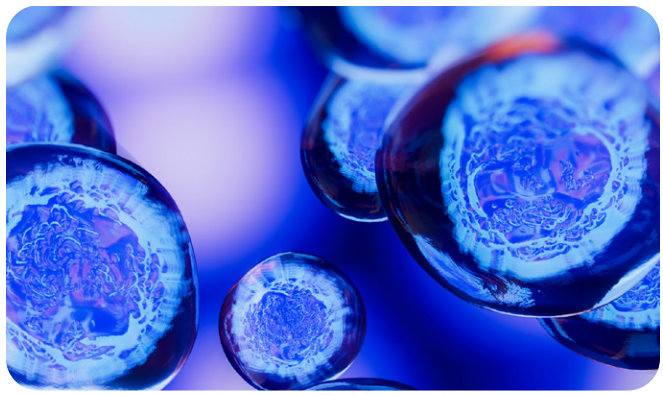
Important notes: *We strongly recommend HyClone™ Characterized Fetal Bovine Serum (U.S. origin, no processing. Cytiva™ Cat. No. SH30071). Please do not use heat-inactivated or irradiated FBS as it may inhibit cell growth.*

Thawing

- Please thaw **only 1 vial** for use to prepare your working stock. Freeze a backup stock of additional vials from the first few passages.

Growth medium and growth rates

- Use only the specified growth medium composition as shown in Table 1 for each cell line. See Table 2 for Cell Culture Materials and Media Components.
- Each cell line grows at a different rate. Refer to average doubling times in Table 1, and set culture conditions and expectations accordingly.



Antibiotics

GFP-expressing cell lines

- Do not use ANY antibiotic at any point with GFP-expressing cell lines. IVISbrite™ 4T1 Red F-luc-GFP (BW128090) and IVISbrite PC3 Red F-luc-GFP (BW133416) as they do not have ANY antibiotic selection resistance and will not survive.

Non GFP-expressing cell lines

- *During the thawing process before cells reach confluence* and again when the cells are banked, the media should be completely antibiotic-free.

  However, once the passaging process has begun, we recommend adding Puromycin to non-GFP-expressing cell lines as noted (see Protocol on page 3 of this document). Though not required, we have found that throughout the passaging process and when plating cells for IVIS assays, the addition of Puromycin as specified encourages the most robust signal.

All non-GFP-expressing IVISbrite cell lines, except for IVISbrite 4T1 Red F-luc (BW124087), can be passaged in medium containing 2 µg/mL Puromycin. IVISbrite 4T1 Red F-luc can be passaged in medium containing 5 µg/mL Puromycin.

- **We do not recommend the use of any antibiotic other than Puromycin and only as specified.**

Viability

- Only % cell density and % confluence, but not % viability, can be determined by microscopic visualization. To assess true cell line viability, a viable cell count must be performed for all cell lines.

Suspended and mixed adherence cell lines

LNCaP, K562, Colo205, and LL/2

- **IVISbrite LNCaP Red F-luc** (BW125055) is a mixture of adherent and semi-adherent cells that requires special culture techniques. Please refer to the IVISbrite LNCaP Red F-luc Tumor Cell Culture Guidelines document available on our website.
- **IVISbrite K562 Red F-luc** (BW124735) is a suspension cell line and does not require trypsinization for passage. When cells reach 80-90% cell density/confluency in suspension, depending on doubling time obtained, simply dilute the culture 1:2 to 1:10 by plating in a larger flask containing fresh, warm medium.
- **IVISbrite Colo205 Red F-luc** (BW124317) and **IVISbrite LL/2 Red F-luc** (BW119267) are mixtures of adherent and suspended cells. When % suspended cell density/ confluency is high and the plate looks full of cells, collect the culture media first to obtain the suspension cells. If adherent cells are loosely attached, directly add trypsin without rinsing with PBS as cells maybe lost with the PBS rinse. If adherent cells are tightly attached, a quick rinse with PBS followed by trypsin treatment is recommended. Neutralize with 2x media. Pool all cells together, do a cell count, and proceed to plate in a larger flask with additional fresh medium containing 2 µg/mL Puromycin.

In all instances where HyClone FBS is mentioned, we strongly recommend HyClone Characterized Fetal Bovine Serum (U.S. origin, no processing. Cytiva Cat. No. SH30071). Do not use heat-inactivated or irradiated FBS as it may inhibit cell growth.

Table 1: IVISbrite Tumor Cell Lines.

Product	Product description	Media composition*	Average doubling time ***
BW124087	IVISbrite 4T1 Red F-luc	RPMI+10% HyClone FBS	14
BW128090	IVISbrite 4T1 Red F-luc-GFP**	RPMI+10% HyClone FBS	14
BW124734	IVISbrite B16F10-Red F-luc	RPMI+10% HyClone FBS	15
BW128444	IVISbrite PC3-Red F-luc	EMEM+10% HyClone FBS	24
BW133416	IVISbrite PC3-Red F-luc-GFP**	EMEM+10% HyClone FBS	24
BW124316	IVISbrite NCI-H460-Red F-luc	RPMI+10% HyClone FBS	16
BW125055	IVISbrite LNCaP-Red F-luc****	RPMI+10% HyClone FBS	60
BW134280	IVISbrite HepG2-Red F-luc	EMEM+10% HyClone FBS	30
BW124577	IVISbrite U87MG-Red F-luc	EMEM+10% HyClone FBS	34
BW134246	IVISbrite GL261-Red F-luc	DMEM+10% HyClone FBS	26
BW128092	IVISbrite HT1080-Red F-luc	EMEM+10% HyClone FBS	22
BW125058	IVISbrite BxPC3-Red F-luc	RPMI+10% HyClone FBS	36
BW124353	IVISbrite HT-29-Red F-luc	McCoy's 5a +10% HyClone FBS	24
BW124318	IVISbrite HCT-116-Red F-luc	McCoy's 5a +10% HyClone FBS	16
BW124735	IVISbrite K562-Red F-luc****	RPMI+10% HyClone FBS	15

Product	Product description	Media composition*	Average doubling time ***
BW124317	IVISbrite Colo205-Red F-luc****	RPMI+10% HyClone FBS	28
BW119262	IVISbrite MCF7-Red F-luc	EMEM+10% HyClone FBS	40
BW119267	IVISbrite LL/2-Red F-luc****	DMEM+10% HyClone FBS	24
BW119276	IVISbrite SKOV3-Red F-luc	McCoy's 5a +10% HyClone FBS	35
BW119266	IVISbrite A549-Red F-luc	RPMI+10% HyClone FBS	22

* Puromycin concentration for passaging, but NOT for thawing, should be 2 µg/mL for all cell lines, except for BW124087 which is 5 µg/mL, and GFP cell lines which should have no puromycin added.

** GFP cell lines (BW128090 and BW133416) do not have any antibiotic selection resistance.

*** Doubling time is an average. Actual doubling times will vary based on culture conditions and handling.

**** Suspended and Mixed cell lines.

Protocol

> **Note:** *IVISbrite LNCaP Red F-luc (BW125055) is a mixture of adherent and semi-adherent cells that requires special culture techniques. Refer to the IVISbrite LNCaP Red F-luc Tumor Cell Culture Guidelines document available on our website.*

Thawing a frozen cell vial

Note: All manipulations of cells must be performed under aseptic conditions. See Table 1 for recommended cell culture media composition and average doubling time.

1. Thaw 1 vial in 37 °C water bath with brisk agitation. Vial should be thawed quickly within 2 min. Decontaminate vial with 70% ethanol prior to placing under cell culture hood.
2. Immediately upon thaw, transfer contents of vial into T-25 tissue culture-treated flask containing 8 mL recommended complete medium (without any antibiotics), which is sufficient to dilute the DMSO contained in the original vial. Medium should be warmed and acclimated in flask in tissue culture incubator at 37 °C/5% CO_2 for 15 minutes prior to cell addition.
3. Gently swirl flask once to mix cells then remove 1 mL (or quantity required) of cell suspension to obtain cell count. Do not pipette cells strongly. DO NOT CENTRIFUGE cell suspension. Incubate flask at 37 °C/5% CO_2 for at least 24-48 hours before disturbing flask.
4. Observe flask under microscope at 24-48 hours. If confluency of cells is 80% or higher, proceed to Passaging Cell Lines section. If not, see below:
   a. If flask is less than 80% confluent but cells are well adhered, gently remove medium and then add fresh warmed medium.
   b. If cells are less than 80% confluent and there is a moderate amount of floating cells, gently transfer supernatant containing floaters to separate flask and add fresh warmed medium to original flask. If any transferred floaters do not adhere to new flask within 3-4 days, discard flask.
5. Continue to incubate the flasks, checking under microscope daily, until they reach 80% confluence before attempting to passage each flask. If necessary, repeat medium changes every 3-4 days during incubation period.

Passaging cell lines

For *in vivo* use we recommend less than 10 *in vitro* passages from original vial. However, split cells at least one time before injecting *in vivo*.

When cells are approximately 80% confluent, passage cells to multiple and/or larger flasks. See Table 3 for information regarding appropriate quantities of Trypsin, PBS for wash/media for neutralization and working volume based on flask size.

1. To passage the cells, gently aspirate and discard supernatant from the flask.

2. Add appropriate quantity (3 mL for 25 cm^2 flask) of sterile, room temperature 1X PBS to the flask. Gently swirl the PBS once over the cells and then aspirate and discard rinse.

3. Add appropriate quantity (1.5 mL for 25 cm^2 flask) of 0.05% sterile, warm Trypsin to the flask and gently swirl to allow Trypsin to coat the plate. Incubate at 37 °C for 1-5 mins until cells detach from the surface of the plate upon examination under microscope.

4. Once the majority of the cells have detached, neutralize the Trypsin by adding the appropriate quantity (3 mL for 25 cm^2 flask) complete medium* and resuspend the cells by gently pipetting up and down 1-2 times.

5. Remove 1 mL (or quantity required) of cell suspension in order to obtain cell count.

6. Transfer appropriate volume of cell suspension into multiple and/or larger flasks in a 1:3 – 1:8 ratio based on surface area. Add complete medium to flask up to the working volume. Incubate for 2-7 days until cells become 80% confluent, changing medium every 3-4 days if necessary.

7. Repeat steps 1-6 for additional passages.

* Puromycin at appropriate concentration may be added to the complete medium at Passage 1 and maintained during subsequent passages (2 µg/mL for all cell lines except for IVISbrite 4T1 Red F-luc, BW124087, which is 5 µg/mL. GFP cell lines do not have any antibiotic selection resistance.)

Table 2: Cell Culture Materials and Media Components.

Material	Manufacturer	Catalog number
T-25 Tissue Culture-treated Flasks	Corning	430639
T-75 Tissue Culture-treated Flasks	Corning	430641U
T-175 Tissue Culture-treated Flasks	Corning	431080
DMEM High Glucose Medium	ATCC	30-2002 (500 mL)
EMEM with L-Glutamine Medium	ATCC	30-2003 (500 mL)
McCoy's 5A with L-Glutamine Medium	ATCC	30-2007 (500 mL)
RPMI-1640 High Glucose with L-Glutamine and HEPES Medium	ATCC	30-2001 (500 mL)
HyClone Fetal Bovine Serum, Characterized	Cytiva	SH30071.03 (500 mL), SH30071.01 (100 mL)
0.05% Trypsin-EDTA (1X)	Cytiva	SH30236.01 (100 mL), SH30236.02 (500 mL)
Puromycin Dihydrochloride	Wisent	400-160-EM (100 mg)

Table 3: Volumes for Cell Passaging based on Flask Size.

Flasks	Growth area (cm^2)	Volume trypsin-EDTA (mL)	Volume PBS rinse/ media neutralization (mL)	Recommended working volume (mL)	Recommended working stage
T-25	25	1.5	3	5 to 10	Initiation
T-75	75	3	6	15 to 25	Initiation or Passage
T-150	150	4	8	30 to 50	Passage
T-175	175	5	10	35 to 60	Passage or Expansion for *in vivo* implantation
T-225	225	6	12	45 to 75	Expansion for *in vivo* implantation

Creating cell stocks

1. When cells have reached 80% confluence, harvest as above for passage except in medium without antibiotics. Centrifuge cell suspension at 1000 rpm for 10 minutes. Resuspend cells at appropriate concentration in ice-cold freezing medium (5% DMSO/95% FBS without antibiotics). Aliquot cell suspension into cryogenic storage vials, then place the cryovials in an insulated freezer box at -80 °C. After 24 hours, transfer frozen vials to liquid nitrogen storage.

2. After a day or two, retrieve one vial for thawing and culture to ensure that the batch of frozen cells retained proper viability.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com