PRODUCT GUIDELINES

In Vivo Imaging Reagents

IVISbrite™ Tumor Cell Culture Guidelines

PerkinElmer 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 PerkinElmer Technical Support Team at (800) 762-4000 or at global.techsupport@perkinelmer.com

IMPORTANT NOTES

We strongly recommend Hyclone Fetal Bovine Serum (GE Healthcare Cat. No. SH300071)

- Please thaw *only one vial* for use to prepare your working stock. Freeze a backup stock of additional vials from the first few passage(s).
- Please see the specified growth medium composition as shown in Table 1 for each cell line.
- Each cell line grows at a different rate. Please refer to average doubling times in Table 1, and set culture conditions and expectations accordingly.
- Previous guides may have recommended heat inactivation of serum. Please note that heat inactivation is not required to achieve optimal growth of these cell lines.
- Do not use ANY antibiotic with GFP-expressing cell lines BW128090 and BW133416 as they do not have ANY antibiotic selection resistance.
- Successive passages of non-GFP expressing cell lines can be achieved with or without antibiotic. Antibiotic in the medium is not required for optimal performance of the cells. However, it is recommended to add antibiotic if potential bacterial contamination while growing cells is a concern in your lab. If antibiotic is to be added, please note that only Puromycin at the correct final concentration should be added (as noted on TDS). Do not use any other antibiotic.
- Please note that only % cell density and % confluence but not % viability can be determined by microscopic visualization of cell lines. A viable cell count must be performed for all cell lines to assess true viability.
- 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 bigger vessel containing fresh, warm media.
- BW124317 and BW119267 is a mixture 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 bigger vessel with additional fresh media.

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Table 1: IVISbrite Tumor Cell Line.

Product	Product Description	Media Composition*	Average Doubling Time ***
BW124087	IVISbrite 4T1 - Red - FLuc	RPMI + 10% Hyclone FBS	14
BW128090	IVISbrite 4T1 - Red - FLuc - GFP**	RPMI + 10% Hyclone FBS	14
BW124734	IVISbrite B16F10 - Red - FLuc	RPMI + 10% Hyclone FBS	15
BW128444	IVISbrite PC3 - Red - FLuc	EMEM + 10% Hyclone FBS	24
BW133416	IVISbrite PC3 - Red - FLuc - GFP**	EMEM + 10% Hyclone FBS	24
BW124316	IVISbrite NCI - H460 - Red - FLuc	RPMI + 10% Hyclone FBS	16
BW125055	IVISbrite LNCaP - Red - FLuc	RPMI + 10% Hyclone FBS	60
BW134280	IVISbrite HepG2 - Red - FLuc	EMEM + 10% Hyclone FBS	30
BW124577	IVISbrite U87MG - Red - FLuc	EMEM + 10% Hyclone FBS	34
BW134246	IVISbrite GL261 - Red - FLuc	DMEM + 10% Hyclone FBS	26
BW128092	IVISbrite HT1080 - Red - FLuc	EMEM + 10% Hyclone FBS	22
BW125058	IVISbrite BxPC3 - Red - FLuc	RPMI + 10% Hyclone FBS	36
BW124353	IVISbrite HT - 29 - Red - FLuc	McCoy's 5a + 10% Hyclone FBS	24
BW124318	IVISbrite HCT - 116 - Red - FLuc	McCoy's 5a + 10% Hyclone FBS	16
BW124735	IVISbrite K562 - Red - FLuc****	RPMI + 10% Hyclone FBS	15
BW124317	IVISbrite Colo205 - Red - FLuc****	RPMI + 10% Hyclone FBS	28
BW119262	IVISbrite MCF7 - Red - FLuc	EMEM + 10% Hyclone FBS	40
BW119267	IVISbrite LL/2 - Red - FLuc****	DMEM + 10% Hyclone FBS	24
BW119276	IVISbrite SKOV3 - Red - FLuc	McCoy's 5a + 10% Hyclone FBS	35
BW119266	IVISbrite A549 - Red - FLuc	RPMI + 10% Hyclone FBS	22

** Optional: Puromycin at a final concentration of 2 ug/mL for all cell lines listed above except for BW124087 which is at 5 ug/mL.*

*** 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.*

***** Suspension cell lines*

Thawing a Frozen Cell Vial

1. Thaw the vial rapidly by gentle shaking in 37 °C water bath by hand. Be careful to keep the cap out of the water. Wipe vial dry.
2. Spray the vial and your gloved hands with disinfectant (70% isopropyl alcohol) and wipe dry. Immediately after, open the vial in the hood and transfer contents to 4 mL of warm, sterile growth media with serum but no antibiotics. Mix gently. DO NOT CENTRIFUGE.
3. Count 1 ml of the total cells and immediately plate the remaining cell suspension into a T25 flask. Incubate at 37 °C, 5-6% CO_2, 100% humidity overnight.
4. Next day, examine the cells under the microscope. If the cells are confluent, continue to instructions below for passaging cell lines.

   If the cells are not confluent:

   a. Aseptically remove the media and replace with 5 mL of the same media warmed to 25 °C- 37 °C.

   b. Continue to incubate the plate(s) for an additional 1 - 7 days with minimal disturbance. Change media every 3 - 4 days until the cells reach 80-90% confluency; only then proceed to passage the cells.

Passaging Cell Lines

1. 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*.
2. When cells are approximately 80-90% confluent, passage cells to vessels with a 1:3 to 1:4 split without antibiotic medium.
3. To passage the cells, remove media and add 5 ml of sterile, room temperature 1X PBS. Gently swirl the added PBS once over the cells and remove the PBS immediately.
4. Next, Add 1 ml of 0.05% sterile, warm Trypsin (approximately 1 mL for T25; 2 ml for T75; 4 ml for T150 and 5 ml for T175) to the flask containing cells and gently swirl to allow trypsin to coat the plate. Incubate at 37 °C for 1 - 5 mins to allow cells to dissociate from the plate.
5. Examine the flask under a microscope to confirm dissociation. Neutralize with 2x medium, and gently re-suspend the cells by pipetting up and down 1 - 2 times.
6. Transfer cells into a bigger flask (T75, T150, T175) at a 1:3-1:7 surface area ratio. Continue to incubate the plate(s) for 1 - 7 days with minimal disturbance. Change media every 3 - 4 days until the cells reach 80-90% confluency; only then proceed to passage the cells.

Creating Cell Stocks

1. When cells have reached 80% confluence, freeze aliquots for 24 hours in -80 °C in 5% DMSO/95% FBS without antibiotics. Transfer frozen vials to LN2 tank after 24 hours.
2. We recommend that you thaw one test vial to check and confirm viability by cell counting and/or culturing.

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