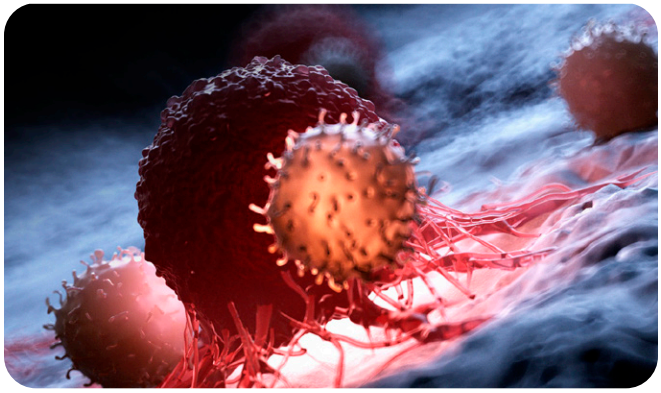
Cell preparation and instructions for *in vivo* injection of IVISbrite tumor cells

Please read and follow instructions in IVISbrite™ Cell Culture Guidelines document prior to working with cell lines. A copy can be downloaded from our website at www.revvity.com

Preparing for *in vivo* injection

1. When cells are in the exponential phase (no more than 70% confluent), collect cells from culturing flask and transfer to 5 mL or 50 mL tube.
2. Spin down cells at 1,000-1,600 rpm for 5 min. Aspirate supernatant media.
3. Wash cells with sterile 1X PBS, then add 1X PBS to tube, and again spin down cells at 1,000-1,600 rpm for 5 min. Aspirate supernatant PBS.
4. Resuspend cell pellet with sterile 1X PBS. The volume of PBS can be from 1 mL to 10 mL. Check cell viability, cell number, and concentration.
5. Once good cellular viability is confirmed, incubate cells in loosely capped tubes in CO_2 incubator for 5-15 min at 37 °C.
6. Gently mixing cells well, add additional 1XPBS as needed to yield final volume and cell concentration for injection. The cell concentration depends on the injection route, tumor cell type, *in vivo* model, and the purpose of *in vivo* study

A final injection volume of 10 µL to 200 µL for each animal should be enough for most *in vivo* studies. Keep the cells on ice until ready for injection. (**Note**: Cell mixture injected into mice should be at room temperature).



Injecting tumor cells *in vivo*

Note: 25g ⅝ to 30g ½ gauge needle is ideal for most *in vivo* injections. For relatively larger cells, a bigger needle is recommended.

Be sure to mix cells well before each injection.

1. Draw cells into the syringe without a needle to prevent cell shearing.
2. Attach the needle to the syringe.
3. Before injecting, flick or invert the syringe to ensure the cells are in suspension.
4. Change needle for each injection.

Do not pass cells through needle more than once since this will shear cells and reduce total cell number.

It is recommended that no more than five mice be injected per syringe.

Concentrating cells in a small volume helps to localize and increase the initial signal *in vivo*.

For research use only. Not for use in diagnostic procedures

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