

## Frequently Asked Questions about Polycaspase Live! Apoptosis Detection Kits

**1. Can I inject more than 40 uL into the animal?** Yes. Simply dilute the stock concentrate with more injection buffer and divide the final volume by 6 (as each vial of reagent contains enough for 6 mice). For example, reconstitute Polycaspase Live with 50 uL DMSO (to form the stock concentrate), and dilute this with 550 uL injection buffer. This yields 600 uL of injection solution - inject 100 uL per mouse.

**2. How many animals can I test with 1 vial?** Each vial of reagent is designed for 6 mice weighing about 30 grams. Larger animals may require more reagent. We have not calculated an exact quantity of Polycaspase Live! needed per gram per animal.

**3. Can I inject Polycaspase Live! intraperitoneal (IP) instead of intravenously (IV)?** We do not recommend IP injections: the reagent simply does not get access to the cells. When injected IP, or at the site of the tissue, the reagent will penetrate and label apoptotic cells nearby through several layers, but it may not permeate all the target tissues. Site-injections may be satisfactory for some experiments. We find that the reagent works best when pumped through the animal in the blood stream.

**4. Why does it need to circulate for 30-60 minutes?** During the circulation period, the reagent enters and exits each cell with the pumping action of the blood system. Polycaspase Live! is cell-permeant crosses the cell membrane automatically. If a cell has active caspases, the reagent will form a covalent bond with the enzyme and will be retained within the cell; it is now simply too big to cross the cell membrane and leave the cell. If the cell does not have active caspases, the unbound Polycaspase Live! will be flushed out of the cell and continue to circulate throughout the body. Polycaspase Live! will be removed from the circulation in about an hour. Circulation periods of longer than 60 minutes are unnecessary. After this time, any caspase-positive cells may begin to break down leading to a lower fluorescent signal. You will see an optimal amount of fluorescence in about 30-45 minutes. Continued circulation of 60 minutes may reduce any non-specific background signals.

**5. How long does it take to clear the body and where does it go?** Polycaspase Live! will clear the circulation in about 1 hour. The reagent may be filtered out through the liver and kidneys, but more research is necessary.

**6. Can I use Polycaspase Live! with other animals besides mice?** Yes. POLYCASPASE LIVE!<sup>™</sup> can be used with other animals. We have used it with mice, rats, chicks, and sparrows.

**7. How do I read the tissues?** With green or red fluorescence. You can examine the tissues directly through a window-chamber models system, or excise the tissues and read with a fluorescence microscope, or quantify cell death with a flow cytometer. We have not found any whole-body imagers that can detect the reagent through the intact animal.

**8. How long will the fluorescence signal last?** Several months, as long as the cells are protected from light. We have frozen brain samples that are still bright after 3 months of storage.

**9. Can I perfuse the animal?** Yes. Sacrifice the animal and prepare tissues as usual: the animal can be perfused; the tissues can be fixed or frozen and thin sections made; or the cells can be put in suspension. Protect from light while handling.

**10. Can I do any other secondary labeling?** Yes. Polycaspase Live! is available in 2 colors: red or green fluorescence so you can do dual-staining studies. Polycaspase Live! works very well with other blue, red, and green labels such as DAPI, GFP, Hoechst, PI and 7-AAD. You can also do a Western with Polycaspase Live!-labeled cells. Protect from light while handling.

**11. Is Polycaspase Live! specific for neurons or cancer?** No. The reagent enters and exits all types of cells. It is specific for caspase enzymes, no matter what type of cell they are in. You can label your specific cell with another marker, such as Nissl for neurons, or a labeled antibody for cancer cells.

**12. Does Polycaspase Live! cross the blood-brain barrier?** Yes. We have looked at rats and sparrows and it enters the neurons without any special treatment.

**13. Does Polycaspase Live! cross the retinal-blood barrier?** Yes. We have looked at mice and it labels retinal glial cells nicely without any special treatment.

**14. How does Polycaspase Live! get into the cell?** Polycaspase Live! is cell-permeant. ICT has optimized the reagent to enter the cell without any lysis or permeabilization steps.

**15. Will I see any background fluorescence?** You might. We have seen a low level of fluorescence throughout the animal, but can still easily and clearly distinguish caspase-positive cells from negative cells. In one case, we had high backgrounds throughout a frozen 20 micron section. The background was easily reduced by exposing the tissue to bright light under the fluorescence microscope for 15 minutes prior to reading the sample. We could still clearly distinguish caspase-positive from negative cells. We generally recommend protecting the cells from light, as the positive signal will eventually bleach out under strong fluorescence.