# Group II Caspase Activity in Bovine Embryos

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## Principle

PhiPhiLux $G_1D_2$ is a fluoroprobe that incorporates the group II caspase-recognition sequence DEVD into a bifluorophore-derivated peptide that mimics the structural loop conformation present in native protease cleavage sites. Group II caspases include caspase 3, caspase 2, and caspase 7. In this molecule, the core peptide, GDEVDGI, is coupled to a molecule of rhodamine on each side of the cleavage site. The two rhodamines interact as a dimer and emit a stable blue-green fluorescence. Cleavage of the substrate disrupts this interaction between rhodamine moieties to result in enhanced green fluorescence (excitation peak 490 nm and emission peak 520). See www.phiphilux.com for more details.

## Materials

PhiPhiLux

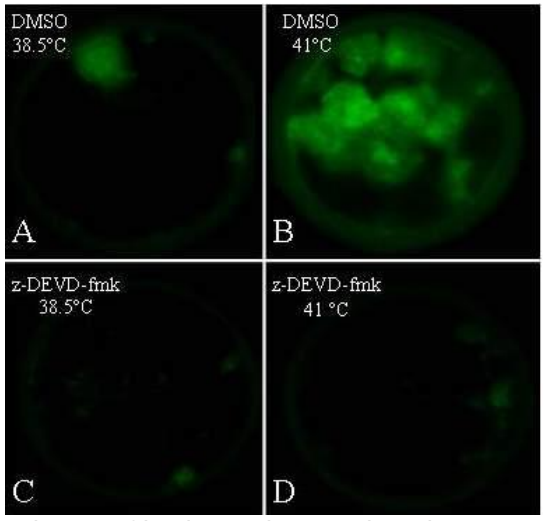
PhiPhiLux $G_1D_2$: OncoImmunin, Inc. (Gaithersburg, MD). Their website has lots of details

Hepes-TALP

Microscope slides: dip the slides in 1:10 poly-l-lysine solution (Sigma P8920) for 2 minutes. Allow the slides to dry. Use a marker to make a circle underneath the slide and then use a hydrophobic pen to make several circular layers on the top of the slide. These layers forms a thick circle that prevents the cover slip from damaging the embryos.

## Procedure

1. Remove the embryos from culture medium and wash three times in 50-μl drops of prewarmed Hepes-TALP
2. Incubate the embryos in 25-μl microdrops of Hepes-TALP containing 5 μM PhiPhiLux $G_1D_2$ at 39°C for 40 min in the dark.
3. Incubate negative controls in Hepes-TALP only. Following incubation, wash the embryos twice in 50-μl drops of Hepes-TALP
4. Place the embryos on poly-L-lysine coated slides (inside the thick circle made with the hydrophobic pen) and mount carefully with a cover slip so it does not crack the embryos.
5. Caspase activity must be determined immediately after the end of treatment (i.e., heat shock) using a Zeiss Axioplan 2 fluorescence microscope with a 453 objective.




Epifluoresence image of bovine embryos cultured at 38.5 or 41 C for 9 h in the presence or absence of the group II caspase inhibitor, D-ZEVD-fmk.

From Paula-Lopes and Hansen, Biochem. Biophys. Res. Commun. 295: 37-42.

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