Fam Caspase 8 and 9 Binding Assay for Embryos

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The FAM caspase binding assay kits from ATCC Corporation can be used to determine amounts of active caspases in cells. The FAM-labeled caspase inhibitor can freely diffuse into the cell. Active caspase irreversibly binds the inhibitor. Upon washing the cells, the amount of fluorescence is proportional to the amount of active caspase in the cell. FAM-LETD-fmk (catalog no. 30-1306) is used to detect caspase 8 and FAM-LEHD-fmk (catalog no. 30-1308) is used for caspase 9. An online manual is available.

Materials

Preparation of 150X FAM Reagent Frozen Aliquot Preparation (Store frozen)
Add 50 µl of DMSO to one vial of lyophilized reagent. Mix until dissolved. Aliquot 10 µl into tubes for later use. Freeze at -20 ^{o}C for 6 months. Solution can be thawed and used twice. Protect from light!

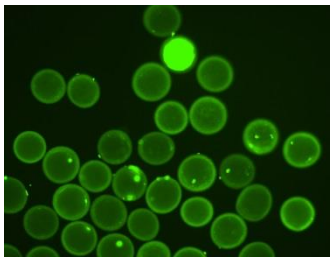
1X Wash Buffer (make on day of use)
Dilute the 10X wash buffer 1:10. For 20 ml: add 2 ml of the 10X wash buffer to 18 ml of deionized water. Mix well and place into the warm oven until the assay is completed. Solution may be re-used for 14 days if stored in the refrigerator.

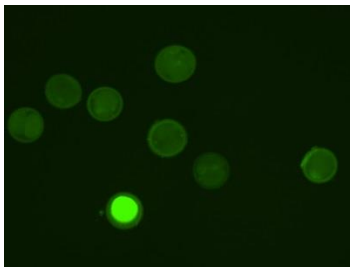
30X FAM Reagent for Assay (make on day of use)
Thaw a 150X aliquot. Make a 30X (1:5 dilution) stock solution by adding 40 µl of HEPES-TALP or 10 mM phosphate buffered saline to the 10 µl aliquot of 150X stock. Protect from light!!!

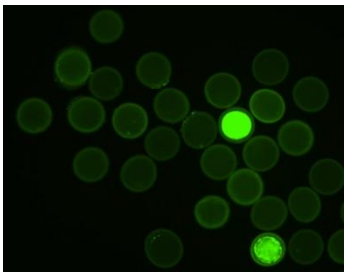
Assay

1) Prepare working solution from the 30X stock. Place 600 µl of HEPES into a microcentrifuge tube and add 20 µl of the 30X FAM Reagent. Mix well and protect from light!!
2) Place a 100 µl drop of the FAM Reagent onto a culture dish (we use 60 mm Petri dishes). Place embryos into the drop and incubate samples at 37 oC in 5% CO2 for one hour.
3) After incubation, wash embryos two times in 100µl drops of 1X wash buffer.
4) Place warm HEPES and embryos on a hanging drop slide and immediately assess caspase activity with a FITC filter.

Below are examples of the caspase assay performed in bovine oocytes. Bright green fluorescence represents caspase activity.







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