FAQs: FLICA® 660 Polycaspase Apoptosis Detection Kit

1. *How many samples can be run with the kits?*

The FLICA® 660 Poly Caspase Assay Kit (KF17361) provides enough reagent to test 7.5 - 15 mL of cell culture samples.

The number of tests to be achieved depends on sample size, application, and preferred working concentration. Following our general recommendations, users may achieve 50 tests per vial for flow cytometry analysis and 25 tests per vial for fluorescence microscopy analysis.

2. *How much working solution is provided per kit?*

FLICA 660-VAD-FMK is supplied as stable, lyophilized reagent, which is reconstituted with DMSO and diluted to working solution with PBS just prior to use. Each vial provides 250 µL of the FLICA working solution that is to be used immediately after preparation.

3. *What are your recommendations for use?*

FLICA 660-VAD-FMK working solution is added to suspension cell samples at a 1:30- 1:60 ratio. The optimal ratio depends on the application and analysis method. Flow cytometry analysis provides the sensitivity to detect FLICA 660-VAD-FMK when used at 1:60. Microscopy analysis may require a higher staining concentration of FLICA 660-VAD-FMK than flow cytometry, so we recommend using the FLICA working solution at 1:30 for this type of analysis. We recommend 300 µL sample sizes, aliquoted from cell cultures grown to a concentration of 2-5 x 10^5 cells/mL. In this manner, 25-50 tests per vial may be achieved. Different sample volumes may be used, which changes the amount of FLICA to be used per sample and alters the number of tests per vial accordingly.

Recommendations for Flow Cytometry: For analysis by flow cytometry, sufficient staining of apoptotic cells may be achieved by using the FLICA 660-VAD-FMK working solution at a 1:60 ratio in suspension cells at 2-5 x 105 cells/mL. E.g., add 5 µL of working solution to a 300 µL aliquot of cells. In this particular example, 50 tests per vial of FLICA 660-VAD-FMK are achieved; actual number of tests will increase or decrease depending on sample size. With its optimal emission peak at 680-690 nm, the signal from far-red FLICA 660-VAD-FMK can be easily read in the FL-4 channel.

Recommendations for Fluorescence Microscopy: This application requires a higher concentration of cells per mL and a higher staining concentration of FLICA® 660-VAD-FMK reagent when compared with flow cytometric analysis methods. To provide an adequate cell density within the field of vision at higher magnifications, the cells should be concentrated by centrifugation to 2-5 x 106 cells/mL just prior to staining with FLICA 660-VAD-FMK. (The initial cell concentration in cultures should be between 2 – 5 x 10^5 cells/mL.) Accordingly, analysis by fluorescence microscopy also requires a higher staining concentration of FLICA 660-VAD-FMK reagent. For this analysis method, use the FLICA 660-VAD-FMK working solution at a 1:30 ratio in the cell suspension, e.g., add 10 µL of working solution to a 300 µL aliquot of cells.
In this particular example, 25 tests per vial of FLICA 660-VAD-FMK are achieved; actual number of tests will increase or decrease depending on sample size.

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4. *How is FLICA different from other caspase detection kits?*

- Cell permeant reagents enable whole cell analysis via imaging and cytometry applications.
- Flexible multiplexing is possible with the use of additional fluorescent dyes or probes.
- FLICA assay kits are used with whole, living cells; no lysis or permeabilization is necessary.
- FLICA is not an ELISA and does not involve the use of any antibodies or substrates. Active caspase enzymes will bind to FLICA 660-VAD-FMK within the living cell, so there will be no interference from pro-caspases or lysing procedures.
- How soon should the samples be read within labeling?
- If cells are not to be read immediately, we recommend fixing the samples with the kit's Fixative and reading the cells within 16 hours, protected from light.

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