

7-AAD Cell Viability Assay Kit

Introduction

The 7-AAD Cell Viability Assay Kit is a kit that uses the fluorescent probe 7-AAD to detect cell necrosis specifically. 7-AAD (7-amino actinomycin D) is a commonly used nuclear probe that binds to DNA and emits red fluorescence. Similar to propidium iodide (PI), 7-AAD is not a cell-permeable probe, so 7-AAD can only pass through cells with damaged membranes and stain their nuclei. But unlike PI, 7-AAD has a narrower emission spectrum, and longer emission wavelengths, leading to less interference to other channels. Therefore, 7-AAD is often used to replace PI in combination with other fluorescent dyes (such as FITC, PE, and APC).

This kit is suitable for the detection of apoptosis and necrosis in combination with Calcein AM, Annexin V-FITC, Annexin V-PE, and so on. This kit can be detected by fluorescence microscopy, flow cytometry or other fluorescence detection instruments after staining. This kit can also be used in flow cytometry to exclude nonviable cells.

Components and Storage

Components	K2235-200 T	K2235-1000 T
7-AAD (20X)	1 mL	5 mL
Staining buffer	50 mL	250 mL

This product should be stored at -20°C, stable for 1 year. 7-AAD (20X) should be stored at -20°C away from light, and avoid repeated freeze/thaw cycles.

Protocol

- 1. Preparation of 7-AAD working solution:** An appropriate amount of 7-AAD (20X) is diluted in staining buffer at a ratio of 1:20 to make a 7-AAD working solution. The working solution is unstable, any unused working solution should be discarded after use.

***Note:** The dilution ratio of 7-AAD (20X) can be adjusted between 10-100 fold depending on the specific experiment.

- 2. 7-AAD staining:**

The following are routine testing steps for adherent / suspension cells. In addition, adherent cells can be detected directly in the dishes or plates, and the staining procedure is similar to the procedure described below, but this may be possible that some apoptotic or necrotic cells are undetectable because they are not adherent.

- 1) **Cell preparation:** For adherent cells, collect the medium for cultured cells and transfer it to a suitable centrifuge tube. Wash cells in PBS one time and add an appropriate amount of trypsin to digested cells. After digestion, add the transferred medium to gently pipette the cells down, transfer cells to a centrifuge tube, and centrifuge at 800 rpm for 5 min. For suspension cells, harvest cells and directly centrifuge the cells at 800 rpm for 5 min.

***Note:** The transferred medium is added after digestion to retain cells that have been suspended due to apoptosis or necrosis, and to prevent the loss of these cells from affecting the experimental result.

- 2) **Washing:** Discard the supernatant, and wash cells in PBS one time. Then resuspend cells in PBS to make the cell counting.
- 3) **Staining:** Centrifuge to discard the supernatant, resuspend cells with 7-AAD working solution and adjust cell density to 1×10^6 cells/mL. Incubate at 37°C protected from light for 5-15 min.

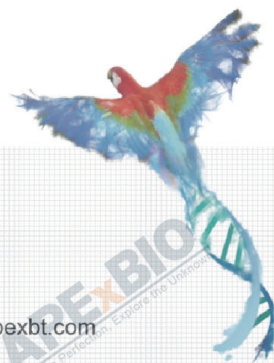
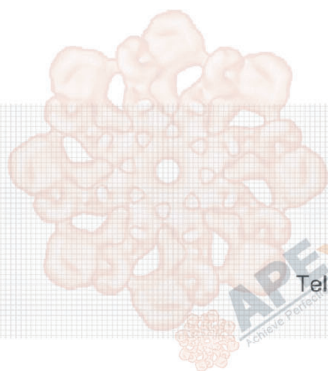
***Note:** The incubation time can be adjusted according to the specific experiment. When using flow cytometry for detection, samples only containing staining buffer need to be prepared as a negative control.

- 4) **Washing:** After incubation, centrifuge to discard the supernatant. Then wash cells in PBS 2-3 times.
- 5) **Detection:** After washing, resuspend the cells with an appropriate amount of staining buffer. Then detect the fluorescent signal by flow cytometry or fluorescence microscope (Ex/Em: 546/647 nm).

***Note:** For living cell staining, because 7-AAD is toxic to cells, detection should be performed as soon as possible after staining.

Note

1. Fluorescent probes are easy to quench, please protect them from light during storage and use.
2. For living cell staining, because 7-AAD is toxic to cells, detection should be performed as soon as possible after staining.
3. If there is bacterial or fungal contamination of the cells, it may seriously affect the detection of this kit.
4. 7-AAD is a potential carcinogen, please pay attention to protection when handling.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.



APEx BIO Technology

www.apexbt.com

7505 Fannin street, Suite 410, Houston, TX 77054.

Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com

