Apoptosis Detection Kit (Luciferase-based High Throughput Screening)

Cat#: AL100 or ExoQA

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User Instruction

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Store AnnexinV-luciferase and Substrate at -80°C and 10x binding buffer at -20°C on receipt

High-Throughput Screening of Apoptosis

Apoptosis, programmed cell death, is a normal activity of multicellular organisms. Insufficient or excess of apoptosis is usually observed in many human diseases such as cancers, autoimmune disorders, diabetes, Alzheimer's, organ and bone marrow transplant rejection etc. Current methods detecting apoptosis include fluorescent dye-based staining approaches, such as ethidium bromide and acridine orange (EB/AO), DAPI (4; 6-diamidino-2phenylidole), Hoechst staining, DNA ladder, TUNEL (Terminal deoxynucleotidyl transferase mediated dUTP nick end Labeling), and enzyme-based approaches, such as, Caspase-3/7 activity. However, these methods are involved in multiple steps, expensive and time-consuming, and even false-positive as well as detecting the late stage of apoptosis. To overcome these shortcomings of current methods, we developed a high throughput screening to quantify apoptosis of adherent or suspension cells in a 96-well plate format, which is suitable for large-scale sample screening of apoptosis.

Phosphatidylserine (PS) in living cells, is primarily restricted to the inner membrane leaflet. However, PS would be translocated from the inner leaflet of the plasma membrane to the outer leaflet during the early to middle stages of apoptosis. Therefore, its surface translocation makes it a potential indicator for the early apoptosis.

Annexin V, one of family of the Annexins, binds phosphatidylserine (PS) in a Ca^{2+}-dependent manner. The commercially available dye-labeled Annexin V, is widely used to quantify apoptosis by FACS, as the standard protocol in cell biology research, due to its binding cell-surface PS with high specificity and affinity. However, it was difficult to do high throughput screening of apoptosis using dye-labeled annexin V FACS. We thus over-expressed Annexin V protein and luciferase as the fused protein (Annexin V-Luciferase, Annexin V-luc). The fused Annexin V-luciferase can be used to monitor apoptosis with high throughput manner. The luciferase signal gave a wider dynamic range than other methods. A workflow of high throughput screening of apoptosis using Annexin V-Luciferase was outlined below.

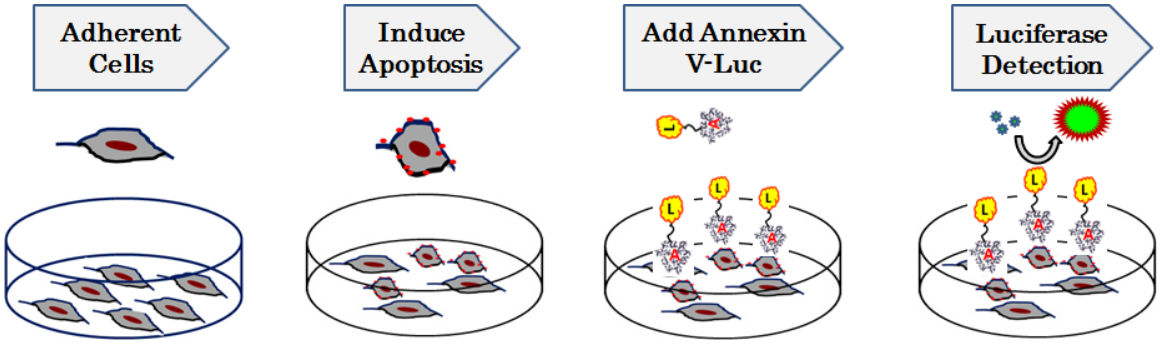

Fig.1. Cross-section from a well of a 96-well microplate illustrating the principle of the apoptosis assay. Adherent cells, induced apoptosis cells, Annexin V-Luc, substrate ad signal were indicated.

Kit Feature and Benefit:

- Detection method: Annexin V-mediated Luciferase Assay;
- Sample type: Living cells (suspension and adherent);
- Species reactivity: Mammalian;
- Kit size: 100 reactions;
- Applications: Detect early/middle stages of apoptosis;
- Simple one step staining procedure in 30 minutes;
- High Throughput and Fast;
- A wide dynamic range of luciferase signal.

Kit components:

- Annexin V-Luciferase protein (store at -80°C).
- 100x Luciferase substrate (store at -80°C).
- 10x Binding Buffer (store at -20°C).

Usage: For Research Use Only! Not For Use in Diagnosis.

Protocol for Apoptosis Detection in Adherent Cells

1. **Seeding Cells**: Cells at the desired cell density should be seeded in a 96-well white or black microplate.
2. **Inducing Apoptosis**: After certain incubation, cells would be treated with your desired apoptosis inducer(s) for certain time to induce apoptosis.
3. **Preparation of 1x Binding Buffer**: Dilute 10x binding buffer with double distilled H_2O into 1x Binding Buffer.
4. **Preparation of Annexin V-luc Assay Diluent**: Add 100 µl of Annexin V-Luciferase protein to 9.9 ml of 1x binding buffer, mix well and keep in ice until use.
5. **Annexin V-luc Binding**: After washing cells with 200 µl of 1x Binding Buffer once, the adherent cells were incubated with 100 µl of Annexin V Assay Diluent at room temperature for 30 min.
6. **Preparing Luciferase Substrate:** Dilute Luciferase Substrate (100x) with plain cell culture media, e.g. DMEM (not provided) without serum, into 1x (e.g. add 100 µl of Luciferase Substrate solution into 9.9 ml of DMEM); Keep the diluted substrate in dark at room temperature for at least 30 min before usage.
7. **Washing:** After incubation of cells with Annexin V-luc for 30 min, remove Annexin-luc solution. The adherent cells would be washed with 200 µl of 1x Binding Buffer Twice. Finally, add 50 µl of 1x binding buffer to cells.
8. **Luciferase Assay**: Luminescence plate reader with automated substrate injector, such as TECAN infinite (TECAN), FlexStation (Molecular Device), was suggested. Inject 50 µl of the diluted luciferase substrate to each well with luminescent setting: integration time (1000ms) and settle time (1000ms). Luciferase activity reflecting cell-associated apoptosis in each well of 96-well would be measured.
9. **Others**: At this point, cells could be used for other assays, such as viability, violet crystal staining etc.

Protocol for Apoptosis Detection in Suspension Cells

1. **Seeding suspension cells**: Cells at the desired cell density should be included in each well of a 96-well plate.
2. **Inducing Apoptosis**: After overnight incubation, cells would be treated with the desired apoptosis inducer(s) for certain time to induce apoptosis.
3. **Harvest Cells**: Cells would be collected by centrifugation at 300g for 10 min.
4. **Preparation of 1x Annexin V Binding Buffer**: Dilute 10x binding buffer with double distilled H_2O into 1x Annexin V Binding Buffer.
5. **Preparation of Annexin V-luc Assay Diluent**: Add 100 µl of Annexin V-Luciferase protein to 9.9 ml of 1x binding buffer, mix well and keep in ice until use.
6. **Annexin V-luc Binding**: After washing cells with 200 µl of 1x Binding Buffer once, cells were incubated with 100 µl of Annexin V Assay Diluent at room temperature for 30 min.
7. **Preparing Luciferase Substrate:** Dilute Luciferase Substrate (100x) with plain cell culture media, e.g. DMEM (not provided) without serum, into 1x (e.g. add 100 µl of Luciferase Substrate solution into 9.9 ml of DMEM); Keep the diluted substrate in dark at room temperature for at least 30 min before usage.
8. **Washing:** After incubation of cells with Annexin V-luc for 30 min, remove Annexin-luc solution. The cells would be washed with 200 µl of 1x Binding Buffer Twice. Finally, add 50 µl of 1x Annexin V binding buffer to cells.
9. **Luciferase Assay**: Luminescence plate reader with automated substrate injector, such as TECAN infinite (TECAN), FlexStation (Molecular Device), was suggested. Inject 50 µl of the diluted luciferase substrate to each well with luminescent setting: integration time (1000ms) and settle time (1000ms). Luciferase activity reflecting cell-associated apoptosis in each well of 96-well would be measured.
10. **Others**: At this point, cells could be used for other assays, such as viability, violet crystal staining etc.