	# EnoGeneApo™ Annexin V-EGFP Apoptosis Kit	E1CK|000020909|50

Storage/Stability:	Store the kit at 4℃. Annexin V-EGFP and Propidium Iodide shall not be exposed to light. The kit will remain stable for one year.
	A. Incubation of suspension cells with Annexin V-EGFP 1. Induce apoptosis by a desired method. 2. Collect 1～5x105 suspension cells by centrifugation at 2000 rpm for 5 min, or collect adherent cells by using trypsin without EDTA. 3. Wash cells twice with PBS (centrifugation at 2000 rpm for 5 min) 4. Resuspend cells in 500 µl Binding Buffer. 5. Add 5µl of Annexin V-EGFP and 5µl of propidium iodide (PI), and mix, respectively. 5. Incubate at room temperature for 5～15 min ,away from light . Proceed as follow as C or D depending on method of analysis. B. Incubation of adherent cells with Annexin V-EGFP For adherent cells, there are two methods. a.Enzyme digest 1.Collect 1～5x105 adherent cells by using trypsin without EDTA. After digested, cells were kept in culture medium with serum to prevent trypsin further digest.

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Experimental Methods:	2.The follow procedures are the same as Step A3～A5. b.Directly Observation 1.Culture cells on a coverslip, and induce apoptosis with proper inducer directly. Set negative control. 2.Rinse samples twice with PBS 3.Add 5μl Annexin V-EGFP and 5μl Propidium Iodide into 500μl Binding Buffer, mix. 4.Add the mixed reagent above on the coverslip and make it uniform. 5.Incubate in wet and dark surrounding for 5min. C. Detection by Fluorescence Microscopy 1. Place the stained cells from Step A.5, or Step B.b.5 on a glass slide. Cover the cells with a coverslip. 2. Observe the cells under a fluorescence microscope using a dual filter for FITC and rhodamine. Cells which have been bound with Annexin V-EGFP will show green in the plasma membrane. Cells which have lost membrane integrity will show red (PI) throughout the nucleus and a halo of green staining (EGFP) on the cell surface (plasma membrane). D. Quantification by Flow Cytometry Analyze Annexin V-EGFP binding by flow cytometry (Ex =488 nm; Em =530nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2). Fluorescence Balance: normal cells as the control, balance fluorescence to reduce spectrum overlapping and set the situation of orientation system.