

Detecting apoptosis in human kidney (HK-2) cells

Elizabeth A. Raupach & Lloyd W. Turtinen*

Department of Biology, University of Wisconsin—Eau Claire, Eau Claire WI, 54702



Abstract

Identification of apoptosis and quantification of cell death can be done with a combination of morphological criteria and analysis of DNA. A newer method utilizes an in situ approach to identify cells with damaged DNA due to apoptosis. We established conditions to grow monolayers of HK-2 cells in 8 well chambered slides and established criteria for efficient labeling using different permeability agents and fixation procedures. We are currently investigating whether various fungal antibiotics induce apoptosis in these cells.

What is apoptosis?

Apoptosis, also known as programmed cell death, is the natural process of non-damaging cell suicide. This process can be triggered by a number of external and internal signals and involves a series of biochemical signals that result in cell shrinking, DNA fragmentation, and the dissolution of the cell by budding into vesicles, which is called blebbing.

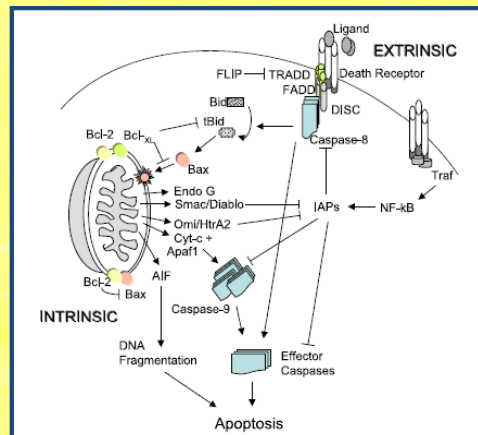


Figure 1. Scheme of extrinsic and intrinsic apoptosis pathways.¹

There are a number of ways to detect the changes in cell morphology and fragmentation of DNA resulting from apoptosis. A very common method to detect DNA fragmentation is to use terminal deoxynucleotidyl transferase (TdT) which adds labeled DNA nucleotides to the ends of the DNA fragments. In this assay we used TdT to add biotinylated nucleotides to the ends of DNA fragments. Then the cells were treated with streptavidin and stained with diaminobenzidine (DAB). This resulted in a dark brown staining of the nuclei of apoptotic cells.

References

- [1] Crighton, D., & Ryan, K. M. (2004) *Biochimica et Biophysica Acta*. 1705: 3-15.
- [2] Gavrieli, Y., Sherman, Y., & Ben-Sasson, S. A. (1992) *J. Cell Biol.* 119(3): 493-501.
- [3] TACS 2 TdT-DAB In Situ Apoptosis Detection Kit Instructions. (2007) Trvigen, Inc.

Basic Protocol

We are using the TACS 2 TdT-DAB In Situ Apoptosis Detection Kit purchased from Trevigen, which is a TUNEL (TdT-mediated dUTP-biotin nick end labeling) assay kit.² We needed to optimize the protocol for use with HK2 cells. The cells had to be fixed to a slide before beginning the assay. The first step of the assay is to permeabilize the cells so the assay reagents can cross the cell membrane. Then the TdT tailing reaction is performed. After tailing, the cells are treated with streptavidin and stained brown with DAB.³

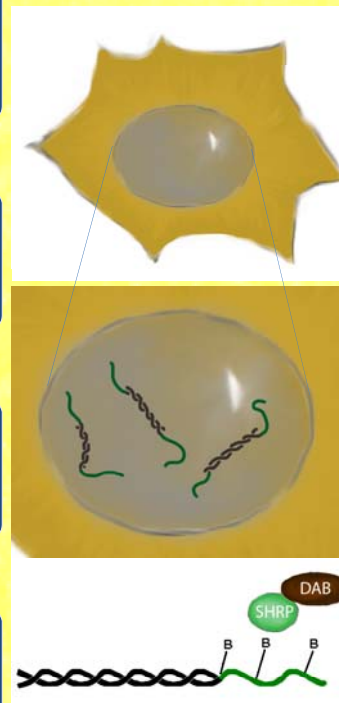


Figure 2. Diagram of assay steps. Top: treating, fixing, and permeabilizing cells. Middle: TdT tailing. Bottom: Strep-HRP binding and diaminobenzidine staining.

Getting cells onto a slide

The assay is designed for use with cells fixed to a slide. In order to perform the assay, we first had to determine growth conditions for the HK-2 cells as well as how to fix them to the slide. We used 8-well chambered slides to grow the cells to about 50-60% confluency. To achieve this, we distributed half of a 7-day culture evenly among the 8 wells and added one more volume of media to each well.

Procedure Modifications

Permeabilization

After fixing the cells to a slide, the first step of the assay is to permeabilize the cells so the assay reagents can enter the cells. Two permeabilization reagents were provided in the kit, Proteinase K and cytonin. We needed to decide which reagent would provide better results as well as the treatment conditions for permeabilization. An initial experiment tested the two reagents side-by-side in otherwise identical sample treatments and the result was darker staining with cytonin. In subsequent experiments, I found that I was able to get even darker staining by allowing the reaction to go for 45 minutes at 4°C, rather than 30 minutes at room temperature as in previous experiments (Figure 3).

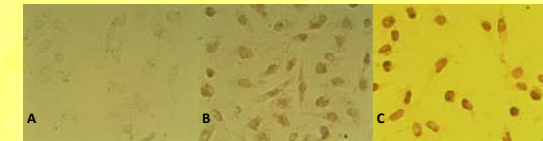


Figure 3. Microscope photos of HK2 cells following apoptosis assay. A. Untreated cells permeabilized with cytonin for 30 minutes at RT. B. Nuclease positive control cells permeabilized with cytonin for 30 minutes at RT. C. Nuclease positive control cells permeabilized with cytonin for 45 minutes at 4°C.

Positive Control

A positive control needed to be established in order to classify cells as apoptotic. The kit provides a nuclease reagent as a positive control. The nuclease is added during the TdT reaction step and mimics apoptosis by digesting the DNA into pieces which can then be labeled during the TdT reaction. However, since the apoptosis pathway is not really induced as it would be with a test reagent, this is not the ideal positive control. Also since the cells would be treated with a test reagent before the apoptosis assay is conducted, adding the nuclease during the TdT reaction in the middle of the assay makes the time frame less comparable for apoptosis. We wanted to find a positive control reagent that would induce the apoptotic pathway and could be used to treat cells at the same time as a test reagent. We chose to use tumor necrosis factor (TNF) for this purpose and found that darker staining indicating apoptosis was found in TNF treated cells than in nuclease treated cells (Figure 4).

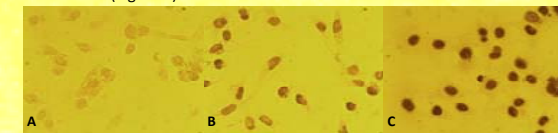


Figure 4. Microscope photos of HK2 cells following apoptosis assay. A. Untreated cells permeabilized with cytonin for 45 minutes at 4°C. B. Nuclease positive control cells permeabilized with cytonin for 45 minutes at 4°C. C. TNF treated positive control cells permeabilized with cytonin for 45 minutes at 4°C.

Conclusions

Permeabilization

- For HK-2 cells, cytonin is a better permeabilization reagent than Proteinase K.

Positive Control

- For HK-2 cells, TNF is a better positive control than nuclease.