

**Altogen  
Labs**

Provider of Preclinical Research Services (GLP/non-GLP) for Drug Discovery  
Efficacy and Pharm/Tox IND contract research studies (clients worldwide)  
100+ Xenograft Models (validated in-house) and IND-enabling Toxicology studies  
100% IP belongs to client, experienced IACUC-regulated barrier facility

# ***Apoptosis Quantification Services***

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# Caspases

- Cysteine-**AS**partic **P**roteases (Caspases) are a group of proteases that have essential roles in programmed cell death.
- A **protease** is a type of enzyme that breaks down peptide bonds in a protein.
- **Cell apoptosis** is "programmed cell death", by which unnecessary or harmful cell/tissue can be relieved of.

# Identification Recommendation

- Identifying cells going through apoptosis can be difficult because other assays used as indicators happen in other types of processes as well.
- Thus, it is recommended to use a variety of different assays to ensure accuracy.

# Cell Cytosol Monitoring

- During apoptosis, changes in the cell cytosol (cytoplasmic matrix) occur. These changes can be tracked and measured. This includes pH, ROS, ion indicators, etc.

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# ***Apoptosis Assays***

- Mitochondria function assays
- Imaging assays
- Microplate assays
- Chromatin condensation
- Annexin V staining
- TUNEL assays

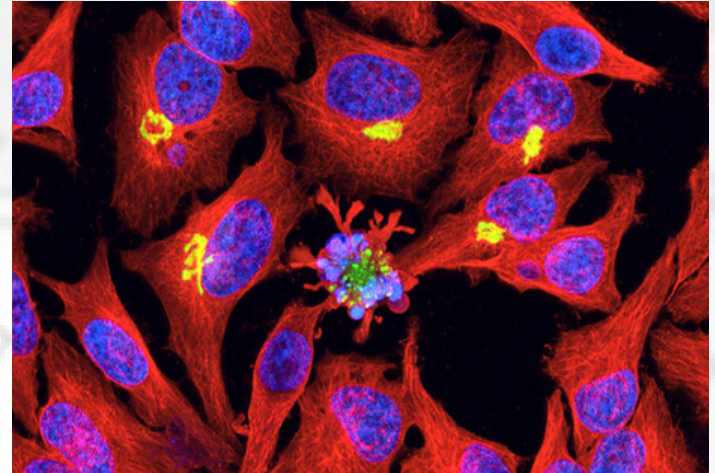
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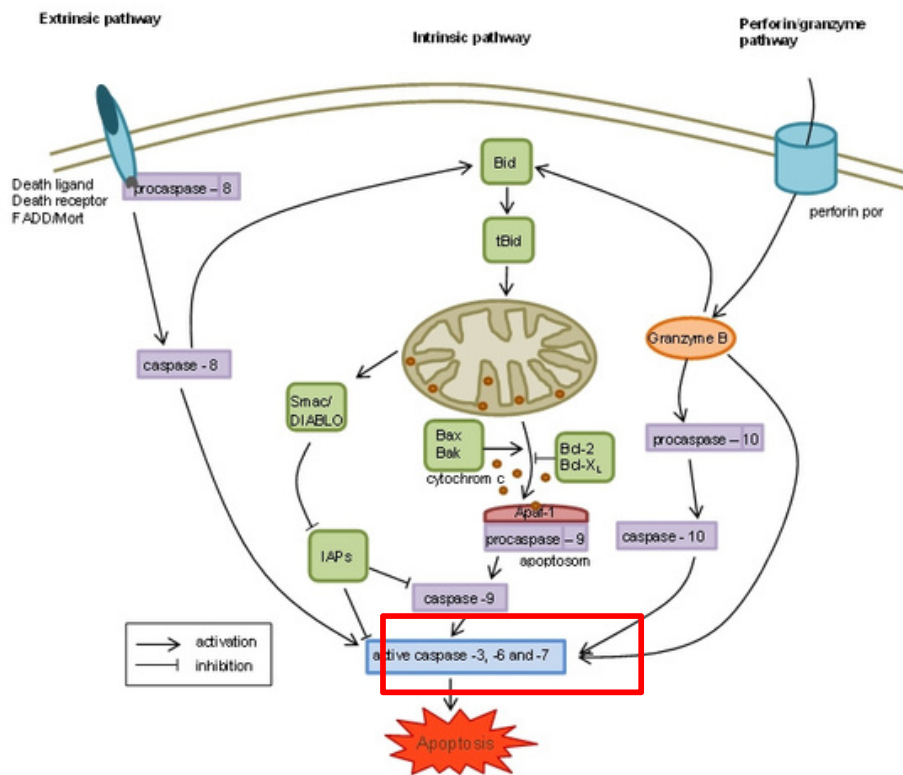
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# Apoptosis Detection through Caspase 3/7 Activity

- Colorimetric/fluorometric/luminescent detection of Caspase activity is a standard detection method for Apoptosis quantification in tissue-specific models.
- Detection of Caspase activity is achieved through the addition of substrate that, once cleaved by the protease, yields a signal.



GFP-expressing HeLa cells undergoing apoptosis.  
<http://www.cell.com/pictureshow/cell-death>



*Signaling cascade leads to activation of Caspase 3/7 which ultimately leads to programmed cell death (Apoptosis)*

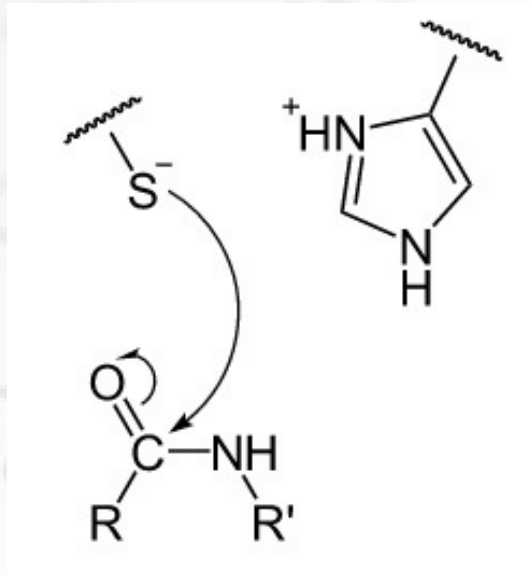
Julita Kulbacka, et. al. (2012). Apoptosis, Free Radicals and Antioxidant Defense in Antitumor Therapy, Antioxidant Enzyme, Prof. Mohammed Amr El-Missiry (Ed.), InTech, DOI:10.5772/50357.

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Caspase 3 cleavage and activation by Caspases 8, 9, and 10 leads to cleavage and activation of Caspases 6 and 7 by Caspase 3. Caspase 7 is cleaved and activated by Caspase 3, 9, and 10.



Nucleophilic attack by an active site Cysteine initiates peptide bond cleavage

[https://en.wikipedia.org/wiki/Cysteine\\_protease#/media/File:Cysteinprotease\\_Reaktionsmechanismus.svg](https://en.wikipedia.org/wiki/Cysteine_protease#/media/File:Cysteinprotease_Reaktionsmechanismus.svg)



Caspase-7 Heterodimer

[https://commons.wikimedia.org/wiki/File%3AProtein\\_CASP7\\_PDB\\_1fj.png](https://commons.wikimedia.org/wiki/File%3AProtein_CASP7_PDB_1fj.png)

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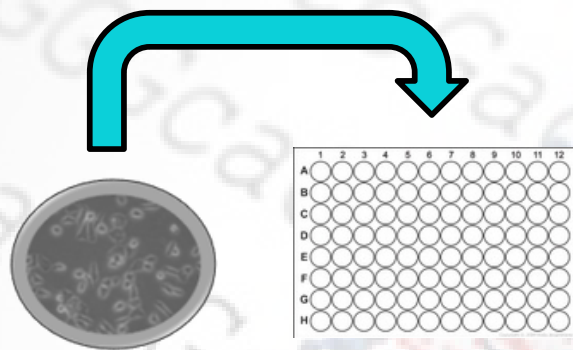


# ***Caspase 3/7 Assay for Apoptosis***

1. Caspase 3/7 substrates are used in a buffer system optimized for caspase activity, luciferase activity, and cell lysis.
2. Log phase cells are transferred to 96-well plates in 200 $\mu$ L of media.
3. Cells are incubated with a compound of interest at a single concentration.
4. Cells are harvested at different time points post-treatment.
5. Caspase reagent is added to the wells resulting in lysis followed by substrate cleavage and generation of fluorescent product.

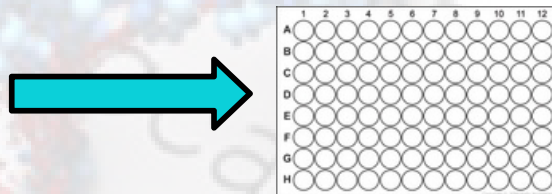
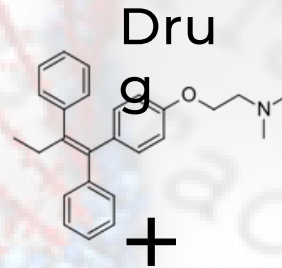
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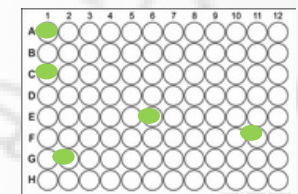
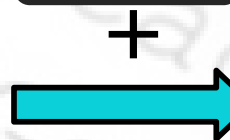
Y. Xu and S. Lu.  
(2014). *Mol. Med. Rep.* 417-421.

1. Log phase cells are transferred to 96-well plate



2. Treat cells with compound of interest

REAGENT

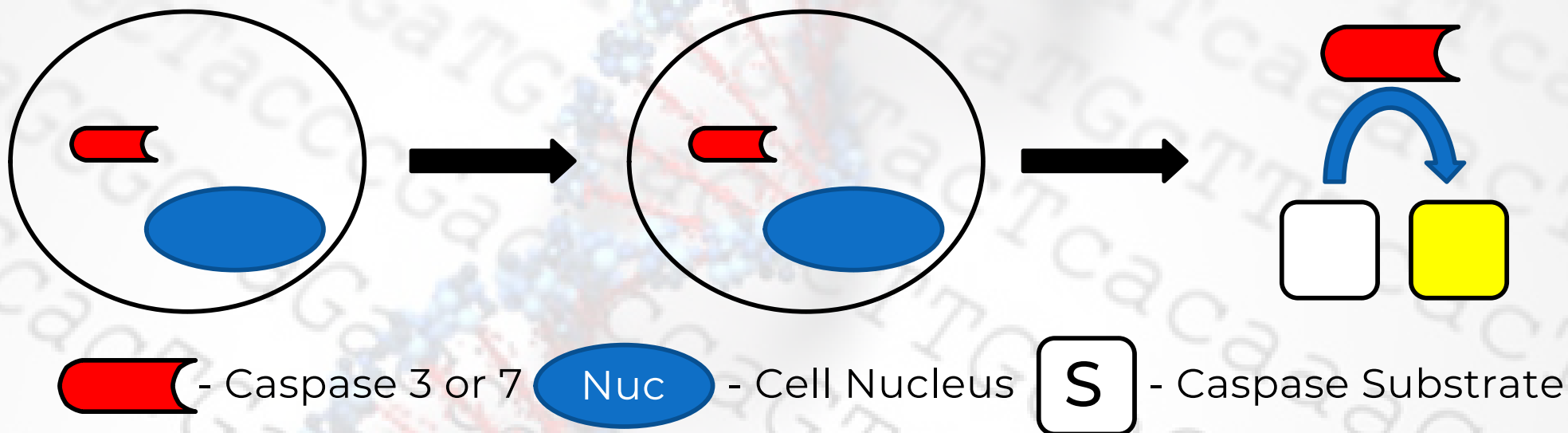


3. Add Caspase reagent and read fluorescence/luminescence

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1. Induction of Apoptosis activates Caspases 3 and 7.
2. After cell lysis, the Caspase reagent is cleaved producing a fluorescent signal that can be measured and quantified.

# Contact Us

- Altogen Labs has a professional staff experienced with Apoptosis assays and is compliant with the FDA's Good Laboratory Practice guidelines.
- Altogen Labs has a vast array of cell lines at its disposal for Caspase 3/7 Apoptosis assays including HepG2 (Hepatocellular Carcinoma) and MCF-7 (Breast Adenocarcinoma).



Sven Hoppe - Fotolia.com

***Contact us to discuss details, timelines, and price!***

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