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

Contact us: info@altogenlabs.com | Read more at AltogenLabs.com
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Caspases

- Cysteine-ASpartic Proteases (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.
**Apoptosis Assays**

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

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

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


Caspase-7 Heterodimer

https://commons.wikimedia.org/wiki/File%3AProtein_CASP7_PDB_1f1j.png
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 200μL 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.
1. Log phase cells are transferred to 96-well plate

2. Treat cells with compound of interest

3. Add Caspase reagent and read fluorescence/luminescence

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.

- Caspase 3 or 7
- Nuc
- Cell Nucleus
- Caspase Substrate
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).

Contact us to discuss details, timelines, and price!