Proliferation and Cytotoxicity Assays

1: Introduction

We will discuss some of the key assays used in cell biology and pharmacology to assess cell viability, proliferation, and cytotoxicity. These assays are pivotal for researchers studying cell growth, drug effects, and cellular responses to various treatments.

When cells are exposed to certain compounds, their ability to survive and proliferate can be affected. By understanding how to measure these processes, we can better evaluate the safety and efficacy of drugs, therapies, or other bioactive substances.

Some of the common assays we'll cover include:

- MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay
- WST (Water Soluble Tetrazolium) assay
- CellTiter-Glo® Luminescent Cell Viability Assay

These assays measure three major aspects:

1. **Cell Viability** – the ability of cells to remain alive under various conditions.
2. **Cell Proliferation** – how quickly cells divide and increase in number.
3. **Cytotoxicity** – the extent to which a substance can cause cell death.

2: MTT Assay – Measuring Viability through Metabolic Activity

Let's start with the **MTT Assay**. This is one of the most widely used methods to assess cell viability and cytotoxicity.

1. **Mechanism**: The MTT assay is based on the metabolic activity of cells. Viable cells contain enzymes, particularly dehydrogenases, which can reduce the MTT reagent into a purple formazan product. This reaction takes place only in living cells, as dead cells lack active metabolic enzymes.
2. **Procedure**:
    - **Step 1**: Cells are plated in a multi-well plate and allowed to grow for a certain period.
    - **Step 2**: MTT reagent is added to each well and incubated for 2–4 hours.
    - **Step 3**: The formazan crystals are solubilized with a solvent such as DMSO (Dimethyl sulfoxide).
    - **Step 4**: The absorbance is measured at 570 nm using a spectrophotometer.
3. **Interpretation**: Higher absorbance indicates greater cell viability and metabolic activity. Reduced absorbance suggests a decrease in cell viability due to cytotoxicity.

Advantages:

- Simple and cost-effective.
- Well-established in many laboratories.
- Suitable for high-throughput screening.

Limitations:

- The assay requires careful handling, as the formazan crystals need to be solubilized.
- MTT reagent can interfere with some treatments, leading to false results.

3: WST Assay – Water-Soluble Tetrazolium Salts

The **WST Assay** is an alternative to the MTT assay, designed to overcome some of the limitations of MTT, particularly the solubilization step. It's a colorimetric assay like MTT but uses water-soluble tetrazolium salts.

1. **Mechanism**: The WST reagent is reduced by mitochondrial enzymes in viable cells, resulting in a color change (usually yellow to orange). The intensity of this color change is proportional to the number of viable cells.
2. **Procedure**:
    - **Step 1**: Cells are cultured in a 96-well plate.
    - **Step 2**: Add the WST reagent to the wells and incubate for a specified time (typically 1–4 hours).
    - **Step 3**: Measure absorbance at around 450 nm using a plate reader.
3. **Interpretation**: The absorbance value correlates with cell metabolic activity. The higher the absorbance, the greater the number of viable cells.

Advantages:

- It's easier and faster than the MTT assay since it doesn't require solubilizing formazan crystals.
- The WST reagent is soluble in water, making it more compatible with different cell types and culture conditions.

Limitations:

- The reagent may give background interference if cells are too dense, requiring optimal conditions for accurate results.

4: CellTiter-Glo® Assay – Measuring ATP as a Marker of Cell Viability

The **CellTiter-Glo® Assay** is a bioluminescent assay that measures ATP levels in cells. Since ATP is a direct indicator of cellular energy and metabolism, this assay provides an accurate and sensitive measurement of viable cells.

1. **Mechanism**: The assay works by using a luciferase enzyme that catalyzes a reaction between ATP and luciferin, producing light (luminescence). The amount of light

emitted is directly proportional to the ATP content in the cells, which reflects cell viability.

2. **Procedure**:
    - **Step 1**: Cells are cultured in a multi-well plate.
    - **Step 2**: Add the CellTiter-Glo® reagent directly to the wells.
    - **Step 3**: Incubate for 10 minutes at room temperature, allowing the reaction to occur.
    - **Step 4**: Measure the luminescence with a luminometer.
3. **Interpretation**: Higher luminescence signals indicate more ATP, which means higher cell viability and metabolic activity.

Advantages:

- Extremely sensitive, even for low cell numbers.
- Simple, with no need for washing or separating steps.
- Can be adapted for high-throughput screening.

Limitations:

- Requires a luminometer, which can be more expensive than a standard spectrophotometer.
- Can be affected by substances that interfere with ATP production.

5: Comparing the Assays – Which One to Choose?

Now, let's compare the three assays we've covered: MTT, WST, and CellTiter-Glo®.

Feature	**MTT Assay**	**WST Assay**	**CellTiter-Glo® Assay**
Type of assay	Colorimetric	Colorimetric	Bioluminescent
Mechanism	Reduction of MTT to formazan	Reduction of WST to a color change	ATP-luciferase reaction
Sensitivity	Moderate	Moderate	High
Ease of use	Requires solubilization of formazan	Direct measurement of color change	Simple, no washing required
Throughput	Moderate	High	Very high
Limitations	Crystal solubilization and background interference	Can be affected by high cell density	Requires a luminometer

Which one to choose?

- If you need high sensitivity and are equipped with a luminometer, the **CellTiter-Glo®** assay is ideal.
- If you are looking for a simpler, cost-effective assay with moderate sensitivity, **MTT** or **WST** assays may be a better choice.

6: Applications of Proliferation and Cytotoxicity Assays

These assays are used in a variety of research areas:

- **Drug Discovery**: Screening potential drug candidates for cytotoxicity and efficacy.
- **Cancer Research**: Evaluating the effects of chemotherapeutic agents on cancer cell lines.
- **Toxicology**: Assessing the toxicity of environmental or industrial chemicals.
- **Immunology**: Studying the effects of immune modulators on cell growth and survival.

7: Conclusion

In conclusion, proliferation and cytotoxicity assays like MTT, WST, and CellTiter-Glo® provide valuable insights into cellular health, metabolism, and response to treatments. Choosing the right assay depends on your experimental design, required sensitivity, and available resources.

When performing these assays, ensure proper controls are used, and always interpret the results in the context of your experiment. Thank you for your attention, and I hope this gives you a clear understanding of how to assess cell viability, proliferation, and cytotoxicity in your research!