IN VITRO BIOCOMPATIBILITY TESTING

iFyber specializes in screening new biomaterials, medical devices and investigational compounds for potential compatibility issues with mammalian biological systems.

We are focused on implementing high throughput, repeatable, quantitative *in vitro* assays that are relevant to the proposed final use of the technology. We provide our clients with solid foundational data from which to plan their future product development efforts.

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iFyber is a preclinical contract research organization offering customized services to companies that operate at the interface of chemistry, microbiology and materials science. iFyber is unique. We pride ourselves on providing access to top scientists and creatively solving problems with quick turnaround times.

THINK OF iFYBER AS:

- Consultants with a laboratory to back up ideas with data
- An academic lab, solving R&D problems on corporate or start-up timelines
- A testing lab that develops new methods tailored to clients' products and services
- An extension of your R&D lab

MTT ASSAY:

ISO 10993-5 BIOLOGICAL EVALUATION OF MEDICAL DEVICES—TESTS FOR *IN VITRO* CYTOTOXICITY

WHAT IS IT?

The MTT assay provides quantitative assessment of cell proliferation by measuring the activity of mitochondrial enzymes that reduce the tetrazolium salt, MTT, to formazan crystals that are solubilized with acidified isopropyl alcohol. The resulting purple solution is quantified spectrophotomerically at 570 nm. Standard intra-assay controls include vehicle-only (usually cell media) and a relevant mammalian cell toxin to demonstrate appropriate test system response.

WHY USE IT?

MTT is widely regarded as the go-to assay for *in vitro* cytotoxicity testing, and is used regularly in industrial and academic R&D laboratories. The MTT assay has been demonstrated to be a relevant predictor of *in vivo* biocompatibility performance, highlighting its usefulness as a screening assay for new biomaterials or investigational compounds. Using ISO as a guideline, a material is considered cytotoxic if cell viability falls below 70% of untreated control cells.

For more information on iFyber's services: www.ifyber.com | info@ifyber.com | 607.330.2307

LIVE/DEAD STAINING:

WHAT IS IT?

The LIVE/DEAD assay is used to determine both cellular membrane integrity and cytosolic enzymatic function in response to a test article. Assay functionality is based on the ability of healthy cells to exclude ethidium homodimer-1, and at the same time cleave the membrane permeable, non-fluorescent calcein AM to calcein through functional cytosolic esterase activity, yielding green cytoplasmic fluorescence. Membrane-compromised cells are permeable to ethidium homodimer-1, which binds to nucleic acids resulting in nuclear-localized red fluorescence.

WHY USE IT?

Unlike most standard cytotoxicity assays, the LIVE/DEAD assay enables concurrent determination of two key aspects of cellular health: membrane permeability and enzymatic function. Healthy cells show green fluorescence, whereas dying or dead cells appear red. Quantification can be carried out by analyzing images that are captured using fluorescence microscopy methods or by using a fluorescence plate reader for high throughput purposes. As such, it provides more detail to the client and can help guide further focused testing.

TUNEL ASSAY:

WHAT IS IT?

Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) is used to identify DNA fragmentation, a terminal hallmark of both apoptotic and necrotic cells. Modified dUTPs are incorporated by the enzyme TdT at the 3'-OH ends of fragmented DNA. The modified dUTPs can be fluorescently labeled allowing quantification of apoptotic/necrotic cell number. This assay utilizes a double staining approach where total DNA appears blue and fragmented DNA appears red.

WHY USE IT?

If a test article demonstrates cytotoxicity as determined by MTT or NRU assays for example, the TUNEL assay is a common approach used to determine whether a cytotoxic response is due to induction of necrotic or apoptotic mechanisms. Specifically, the TUNEL assay probes for the presence of fragmented DNA, a terminal indicator of imminent cell death.

BRDU ASSAY:

WHAT IS IT?

The BrdU assay is based on the fluorometric detection of the thymidine analogue BrdU (bromodeoxyuridine), upon its incorporation into genomic DNA during cell proliferation. BrdU may then be detected using anti-BrdU antibodies, allowing assessment of the population of cells that are synthesizing DNA. This provides an indication of cell proliferation rate and a measurement of the number of cells in S phase of the cell cycle.

WHY USE IT?

If a test article demonstrates cytotoxicity as determined by MTT or NRU assays, the client may want to know more. One of the first aspects of cellular function to investigate is DNA replication. The BrdU assay enables direct comparison between the proliferative status of cells treated with the test article and control cells. A reduction in BrdU uptake by treated cells indicates that DNA replication, and thus cell proliferation, is being negatively impacted.

OTHER AVAILABLE BIOCOMPATIBILITY ASSAYS

- Bright Field Microscopy
- Neutral Red Uptake
- Trypan Blue Exclusion
- Bacterial Reverse Mutation Assay
- Platelet activation
- Hemolysis assay