A TECHNOLOGY GUIDE

for Applying Reagent Dispensing to MTT Assays

INTEGRA

Top Instrument Considerations for an MTT Assay

Since its first application in 1983, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay, also known as the MTT assay, has become the method of choice for determining cell viability and proliferation. Scientists use MTT assays in drug discovery to screen primary therapeutics and evaluate their antigrowth and toxicity effects on cells. During the assay, the yellow-colored, positively charged MTT reagent crosses the cell membrane, where the cell then metabolizes the chemical into formazan crystals, triggering a visible color change to purple. The darker the purple, the more viable cells within the solution (1).

The MTT assay steps are straightforward, but scientists have struggled with the technique's reproducibility (1). MTT assays involve multiple cell and reagent dispensing steps that can quickly compound with increasing multiwell plates, potentially introducing inaccuracies and experimental variability. Additionally, while the MTT assay is safer than other cell viability assays that use radioactive or intercalating agents, MTT can become toxic to cells in high concentrations (1). Researchers should therefore conduct preliminary tests to identify the ideal cell density and MTT concentration for different cell types (1). Using a reagent dispenser for optimization tests, and for automating tedious dispensing steps across multiwell plates, eases the burden on researchers and ensures experimental consistency and reproducibility.

When automating reagent dispensing for an MTT assay, researchers should consider the following instrument needs.

1. Flexibility

To develop new therapeutics, researchers screen leading drug candidates across hundreds to thousands of cells in multiwell plates. The MTT assay was the first homogenous cell viability assay developed for high throughput screening in a 96 well plate (2). Whether using a handheld manual pipette or an electronic multichannel pipette, aliquoting cells and reagents across a 96 well plate is prone to user fatigue and error. A simple reagent dispenser, such as INTEGRA's WELLJET reagent dispenser, automates routine pipetting tasks across different multiwell plate formats from 6 to 1536 well plates. To accommodate different experimental needs, the WELLJET uses 8 or 16 channel dispensing cassettes capable of aliquoting volumes from 0.5 µl to 10 ml. Researchers can even control the dispensing speed of different liquids, such as those that contain cells, to maximize cell viability and meet the dispensing requirements for different reagents. However, the instrument is not merely a blind pipettor. Researchers can leverage its intuitive software to define and save custom protocols for different plate sizes, expediting repeat experiments, decreasing hands-on research time, and improving experimental reproducibility.

2. Space

Many researchers tend to think that, to get the benefits of automation, they must invest in an automated liquid handler. These large, box-like instruments stretch across laboratory benches and occupy valuable research space. In sharp contrast, measuring in at 20 x 46 x 29 cm, the WELLJET reagent dispenser has the smallest footprint of any machine of its kind in the lab, and accomplishes the same automated pipetting goals as a liquid handler. For research teams working in a high throughput setting and going through more than 25 plates in a day, the WELLJET reagent dispenser also comes in a plate stacker version. Slightly larger at 46 x 46 x 63 cm, the WELLJET dispenser stacker dispenses liquids and stacks plates in a tower. This saves researchers further valuable space by neatly organizing dispensed plates, and maximizes productivity by enabling the high throughout screening of thousands of samples. Both instruments can also easily fit under a laminar flow hood, making them convenient reagent dispensing solutions for biosafety workbenches.

3. Affordability

Instead of pipettes, reagent dispensers rely on reagent dispensing cassettes made of reusable tubing to aliquot solutions. Conventional reagent dispensing cassettes are manufactured using extruded silicone, which can cause variations in the inner diameter of the tubing. To mitigate this variability, manufacturers calibrate each individual channel of the cassette by adjusting the tubing tension during the manufacturing process. Over time, this calibration can be lost, requiring researchers to recalibrate their cassettes or discard them to maintain scientific accuracy. The WELLJET reagent dispenser uses a revolutionary type of dispensing cassette, called the EasySnap™ dispensing cassette, made from injection-molded silicone tubing to ensure that every tube is manufactured to the same dimensions, eliminating channel-to-channel variation and the need for recalibration. This makes EasySnap cassettes less expensive to purchase and use over time, saving valuable research funds.

4. Usability

Reagent dispensers are also easy to use. The WELLJET reagent dispenser features a large display screen and an intuitive user interface that makes programming dispensing protocols simple. Researchers can effortlessly load the EasySnap dispensing cassette into the instrument without worrying about breaking or incorrectly placing it. EasySnap dispensing cassettes also come with a radio frequency identification (RFID) tag that allows researchers to track dispensed volumes. The EasySnap RFID tag keeps researchers informed with dispensed volumes and notifies the user when it is time to change the cassette.

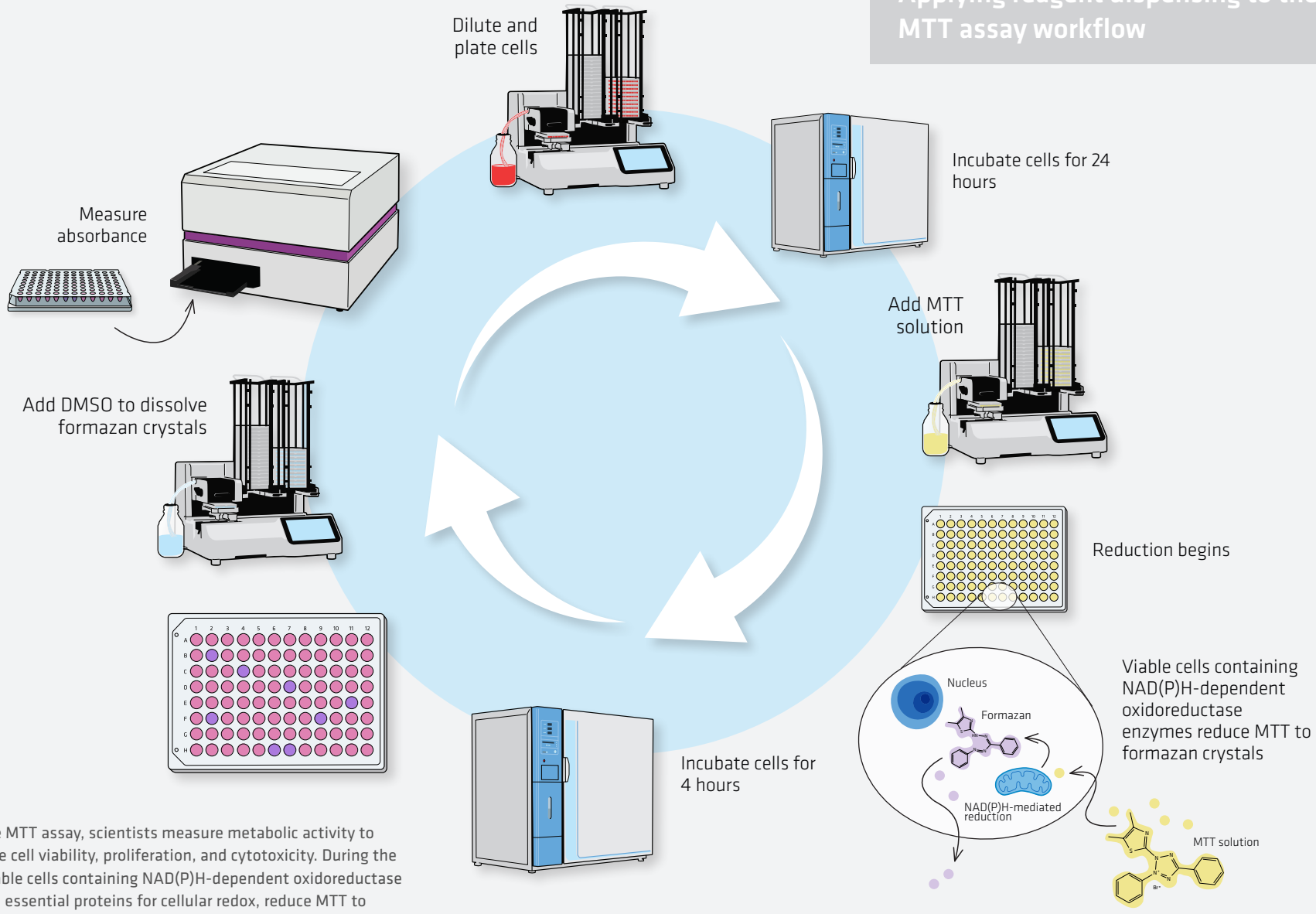
The real value

In addition to the affordability of the WELLJET reagent dispenser, the biggest cost saving advantage of using a high quality reagent dispenser is the optimization of researchers' time. Choosing a reagent dispenser to aliquot liquids across multiwell plates increases experimental reliability and accuracy, and frees researchers to perform more complex experimental tasks, analyze data, and make valuable discoveries sooner.

References

1. Ghasemi, M., Turnbull, T., Sebastian, S., & Kempson, I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *Int J Sci* **22**(23), 12827 (2021).
2. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Meth* **65**, 55-63 (1983).

Applying reagent dispensing to the MTT assay workflow



Using the MTT assay, scientists measure metabolic activity to determine cell viability, proliferation, and cytotoxicity. During the assay, viable cells containing NAD(P)H-dependent oxidoreductase enzymes, essential proteins for cellular redox, reduce MTT to formazan crystals. This reduction changes the solution from yellow to purple.

Essential Materials

Material	Description
WELLJET dispenser	INTEGRA's WELLJET reagent dispenser and dispenser stacker perform multiple dispensing steps in the MTT assay workflow. Using the WELLJET, researchers can seed cells gently in the appropriate concentration. In later steps in the MTT assay, the WELLJET dispenser coordinates cell culture medium, MTT solution and DMSO dispensing across multiple multiwell plates.
EasySnap dispensing cassette	The WELLJET's EasySnap dispensing cassettes use molded silicone tubing with consistent dimensions, ensuring that accurate volumes are dispensed across channels and experiments. EasySnap dispensing cassettes come in 8 and 16 channel configurations for maximum protocol flexibility.
Cell culture medium	Researchers should use the ideal cell culture medium for their cell type of interest to optimize cell viability.
MTT solution	At the start of the assay, researchers dilute MTT in PBS. The solution forms a characteristic yellow color. The mitochondria in living cells metabolize MTT into formazan crystals. As this occurs, the solution turns purple.
DMSO	After incubation with MTT and removal of the cell culture medium containing residual MTT, dimethyl sulfoxide (DMSO) is added to the cells, which dissolves the newly formed formazan crystals.
Plate reader	A multiwell spectrophotometer or plate reader measures the absorbance at 500-600 nm for each well. The higher the absorbance or darker the solution, the greater the number of viable, metabolically active cells are present in each well.

Expert Advice: Navigating Reagent Dispensing

For assays such as the MTT assay that require cell and reagent aliquoting across numerous multiwell plates, precise and consistent reagent dispensing is critical to achieving accurate and reproducible results.

GERALD POSCH, a biotechnologist and product manager with INTEGRA Biosciences, specializes in optimizing the often overlooked but significant details that affect experimental success. Posch earned his master's degree and his PhD from the University of Natural Resources and Life Sciences, Vienna, where he investigated enzyme kinetics, characterized bacterial cell surface properties, and studied bacterial glycosylation, the subtle post-translational addition of sugar moieties to proteins that affect protein dynamics. Posch applied his expertise in glycosylation engineering to vaccine development during his postdoctoral research at the University of Alberta and in the biopharmaceutical industry for five years, before transitioning to INTEGRA Biosciences. Using his eye for detail and talking to researchers on the forefront of drug discovery, Posch and the INTEGRA team developed the WELLJET reagent dispenser as an innovative solution to help ease the tedious pain points of reagent dispensing for common assays, such as the MTT assay.

How does using a reagent dispenser improve MTT assay data?

Dispensing cells and the different solutions that make up an MTT assay needs to be very consistent, which might not be the case if one sets up this assay by hand using simple pipetting. Having an automated dispensing solution helps data reproducibility and consistency. The WELLJET reagent dispenser uses a peristaltic pump system that propels the liquid forward via a rotor integrated into the pump unit of the dispenser. Unlike other reagent dispensers, the WELLJET reagent dispenser uses the EasySnap cassette, which is made from injection-molded silicone to ensure that every tube in the cassette has the same dimensions. The EasySnap cassette design, coupled with the peristaltic pump, provides consistent reagent dispensing across cassette channels, meaning that researchers can rest assured that the same correct volume is applied to each well of their multiwell plate. This eliminates well-to-well variability and safeguards experimental accuracy. The liquid within the silicone tubing doesn't interact with the instrument or pump parts, protecting experimental sterility. The WELLJET can aspirate liquids from different source vessels, which can be anything from bottles to flasks to tubes, and then dispenses the liquid across any size multiwell plate from a 6 well to a 1536 well plate. This allows researchers to tailor their reagent dispenser to meet the unique needs of different experiments and experimental steps.

Changes in cell viability could affect MTT assay results. How can using a reagent dispenser help optimize cell viability?

The main advantage is that it's a very gentle process. The cells are pumped through the silicone tubing at very low shear forces, which is ideal for fragile cell lines. We have two types of dispensing cassettes, a large bore dispensing cassette and a small bore dispensing cassette. The large bore dispensing cassette not only has a wider bore size for the silicone tubing, but also a larger orifice for the dispensing nozzle. If someone is not yet experienced with reagent dispensing because they've always used pipettes, and they want to start dispensing cells, then the first recommendation we give is to use the large bore dispensing cassette with the wider orifice, just to make sure their cells will be happy, especially if the sensitivity and fragility of the cells is unknown. But we have data from customers who have successfully used the small bore cassette with fragile cell lines and at high dispensing speeds.

What other factors should researchers consider when dispensing cells?

It really depends on the cell type for troubleshooting. Dispensing speed is also important. We have three different speed settings for the WELLJET: fast, medium, and slow. We have seen good results with the fast speed setting. If one sees that the cells are not as viable, then slower speeds should be used for further optimization. Also, for other troubleshooting methods, the height of the dispensing module above the plate can have an influence. Finding the right height setting or lowering the dispense height may help researchers get better results for fragile cells.

How should researchers care for their dispensing cassettes to maximize cassette lifespan?

The cassettes are reusable. The standard lifetime in terms of plates that can be dispensed is 2000 96-well plates at 100 microliters. After that, it's recommended to change the cassette. The INTEGRA team supplies sterile cassettes but, to maintain cassettes in optimal condition, it is important to properly clean them after each dispensing

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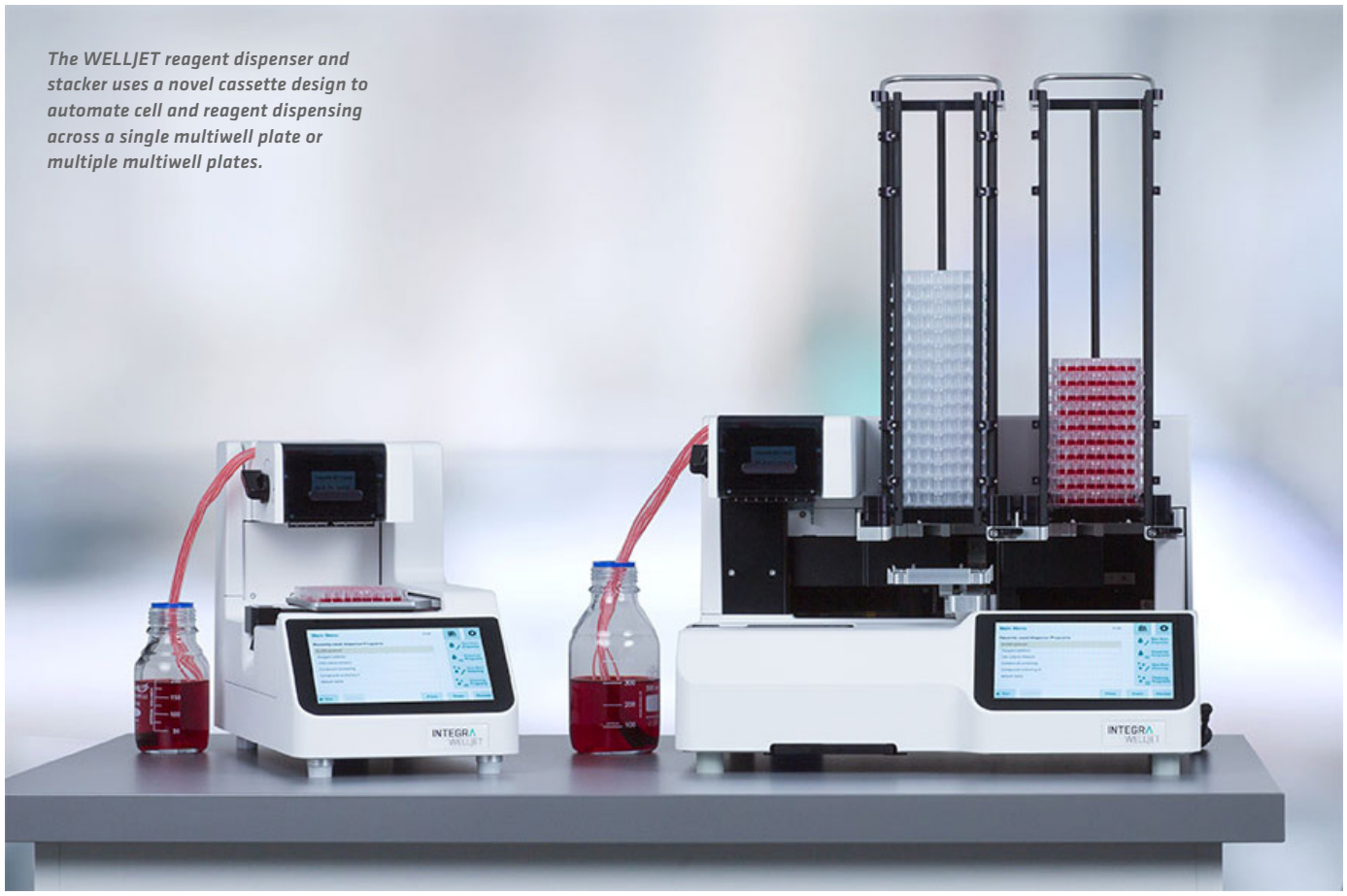
The INTEGRA team around Gerald Posch, a biotechnologist and product manager with INTEGRA, led the development of the WELLJET reagent dispenser, the smallest instrument of its kind that automates tedious cell and reagent dispensing across numerous multiwell plates.

campaign. Dispensed cell solutions, buffers, or compounds contain cells, proteins, and salts, which may cause the cassette to clog, especially at the point of the dispensing nozzles. We recommend that scientists thoroughly wash the cell solution out with deionized water and a mild detergent. They then need to wash out the detergent with water to avoid residual cleaner sitting in the tubing or the cassette when starting the next dispensing campaign with cells. After cleaning, it would be good to rinse the cassette with a disinfectant. Then autoclaving the casettes ensures their sterility. Scientists can set up a cleaning program for these steps, but they need to move the tubing into different source vessels. So, it's not a fully automated process, but we wanted to give customers some help by allowing them to define cleaning programs for the cassettes.



The WELLJET reagent dispenser and stacker uses a novel cassette design to automate cell and reagent dispensing across a single multiwell plate or multiple multiwell plates.

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How can scientists use reagent dispensing in automated workflows?

The dispensing process is automated and, with the dispenser stacker version, scientists can process a whole stack of plates to dispense cells or reagents. In that sense, these instruments are standalone units. However, the WELLJET can also integrate into automated workflows using application programming interface (API) commands. We provide the instrument and API commands that allow an integration company to speak with our system. This triggers directions that allow the instrument to be used in a fully automated system. There are integration companies that combine instruments from different providers to work in concert. For instance, for a full cell-based assay, the WELLJET only dispenses cells or reagents, but a scientist might also want to have an optical readout or incubation steps in between reagent dispensing. This can be handled by a robotic arm and a master software, provided by an integration company. The robotic arm shifts multiwell plates from one instrument to the other, and starts the processes on the instruments. In the end, you can automate a whole assay or a whole drug compound screening platform.

In what other applications can researchers use a reagent dispenser?

Almost every assay requires some sort of reagent dispensing or liquid handling. As such, reagent dispensers can help with almost any experiment. The WELLJET serves plates that meet ANSI/SLAS format, the standard format defined for multiwell plates in terms of plate dimensions and how the different wells are set up. Scientists can start with a single plate and go up to multiple plates, and then, if they need higher throughput, they can switch to the stacker version. Both instruments are designed to have very small footprints to fit inside a biosafety workbench.

The EasySnap dispensing cassette utilizes a revolutionary cassette design made of injection-molded silicone that ensures consistency across dispensing channels.

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You and the INTEGRA team led the development of the WELLJET reagent dispenser from inception to application. What is the most innovative feature of this product?

The most innovative feature for me is the EasySnap dispensing cassette, which uses molded silicone tubings to avoid recalibration, cutting the cost for our customers and ensuring high accuracy and precision across all channels. Also, the large, easy to navigate interface combined with very smooth and precise mechanics, sets the WELLJET apart from other reagent dispensers. We put as much technology as possible into a very compact instrument. That's always the challenge, to make an instrument as compact as possible without compromising on its functionality. We go out and speak with scientists to understand their pain points with existing solutions, and how we can improve those areas with new solutions. We are not aiming to just make another product. We want to make sure that what we develop is also at the forefront of innovation. It's quite a path but, in the end, it's very exciting to talk with customers and hear how it's helping them in terms of efficiency, data generation, reliability, and consistency.