Application Note

INTEGRA

MTT cell proliferation, viability and cytotoxicity assay with the ASSIST PLUS pipetting robot

Introduction

The MTT cell proliferation assay, first described by Mosmann[1], is a commonly used colorimetric assay for assessing cellular metabolic activity. This broadly used, rapid and quantitative cell-based assay can be used to measure cell proliferation, cytotoxicity or cell activation for a variety of cell types. The test is based on the reduction of yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active cells; viable cells contain NADH-dependent oxidoreductase enzymes which reduce the MTT to formazan. The insoluble crystals are dissolved using a solubilization solution with the darker the color of the resulting solution, the greater the number of metabolically active cells.

Key benefits:

- Automating the assay expands sample throughput, maximizes intra- and inter-assay reproducibility as the program is always executed in the same way, and increases walk-away time.
- The compact footprint of the ASSIST PLUS pipetting robot allows processing in a laminar flow cabinet, guaranteeing sterile working conditions.
- The automated process protects the user from toxic fumes by minimizing contact with reagents.
- The use of INTEGRA's divided reagent reservoirs with SureFlo™ anti-sealing array together with Low Retention GripTips allows efficient handling of precious reagents like MTT or cell culture medium with expensive additives.

Step-by-step procedure:

The ASSIST PLUS, together with a VOYAGER 8 channel 125 µl adjustable tip spacing electronic pipette with 125 µl Sterile, Filter, Low retention GripTips, is used to automate the pipetting steps of the MTT cell proliferation, viability and cytotoxicity assay, guiding the user whenever manual intervention is required during the process.

ASSIST PLUS

Prior to starting the MTT assay, 5 mg/ml MTT stock solution with sterile PBS (pH 7.4) should be freshly prepared. Dimethyl sulfoxide (DMSO) is needed to dissolve the formazan crystals. The cells of interest should be seeded at a concentration of 10^4-10^5 cells per well in 100 µl of cell culture medium.

Tip:

- A concentration using fewer cells per well may also be sufficient if very highly proliferating cells are used. However, it is crucial that every well has the same number of cells initially, and replicates and controls should be used. Before the MTT assay, cells should be incubated with the compound to be tested.

The workflow is split across two days. Accordingly, the protocol is divided into two programs that guide the user through the steps of the MTT assay and automate the pipetting steps.

Program 1: Cell incubation with fresh cell culture medium (Cell_incubation)
Program 2: Automated MTT assay (MTT_assay)

Day 1 – Program 1 – Cell incubation with fresh cell culture medium

Experimental set-up

Deck position A: Empty
Deck position B: Dual reservoir adapter containing two 25 ml multichannel reagent reservoirs with SureFlo anti-sealing array.
Deck position C: INTEGRA slanted plate holder (20°) (**Figure 1)** with the seeded cells in a 96 well flat bottom plate (Greiner Bio-One International GmbH, **Figure 2**, green).

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1. Cell incubation with fresh cell culture medium

STEP: Changing cell culture medium before incubation

HOW TO: Load a 25 ml multichannel reservoir onto the left compartment of the dual reservoir adapter and fill with 12 ml of fresh cell culture medium. Place an empty 25 ml multichannel reagent reservoir on the right side of the adapter. Adjust the tilt on the slanted plate holder to 20° by loosening the screw manually, tilting to the 20° indication and tightening the screw again. Place the 96 well flat bottom plate with seeded cells onto the slanted plate holder on deck position C (**Figure 2**). The plate position on the INTEGRA slanted plate holder helps gentle dispensing and complete aspiration of liquids (**Figure 3**).

Select and run the VIALAB program 'Cell_incubation' on the VOYAGER pipette. The pipette removes 100 µl of cell culture medium from each well in column 1, then replaces it with 100 µl fresh medium from the reservoir. This exchange continues column-by-column until the cell culture medium in every well of the entire 96 well plate has been replaced. The process is performed column-by-column to avoid cell drying. Once the pipetting steps are complete, the VIALAB program informs the user to incubate the 96 well plate overnight at 37 °C with 5 % CO_2.

Figure 1: INTEGRA slanted plate holder with a 96 well plate.

Tips:

- THE ASSIST PLUS has a special Cabinet Mode that enables easier installation and control of the pipettes. The pipette holder lowers at the beginning and at the end of the program for easier navigation.
- Pipetting speed can also be altered to aspirate the cell culture medium from the 96 well plate very slowly, avoiding shearing stress to the cells.

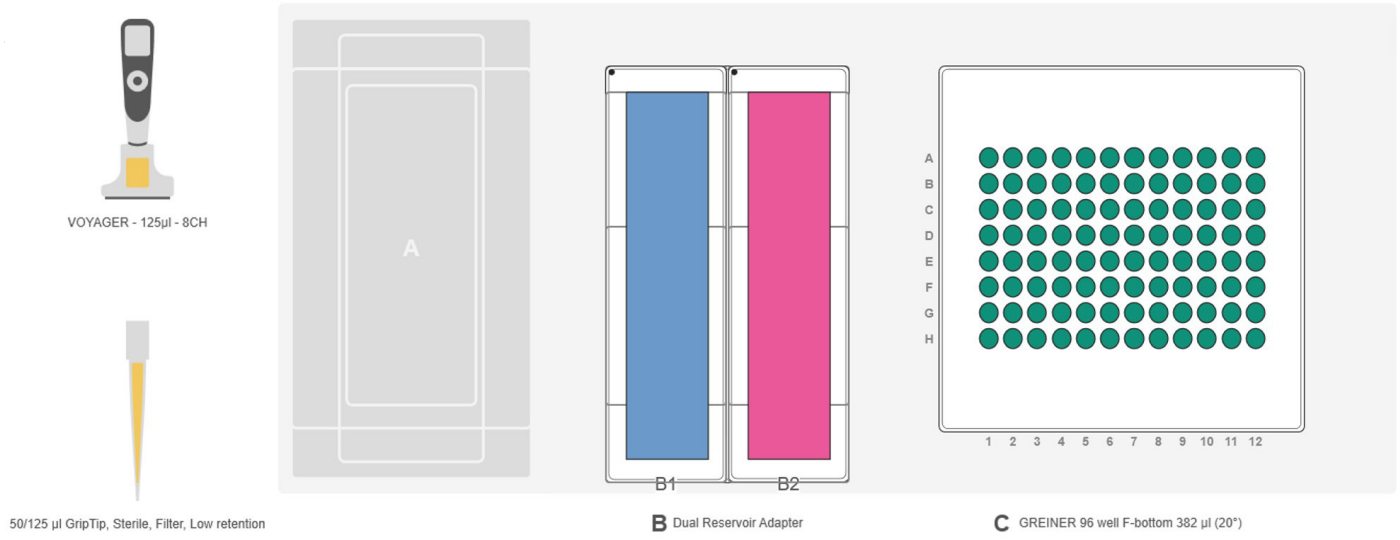
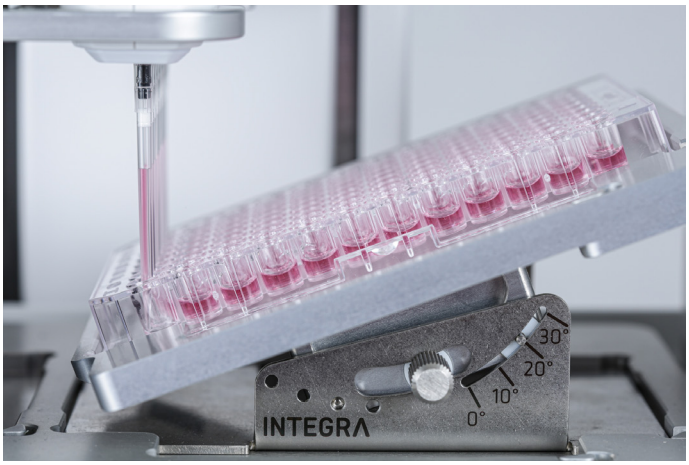


Figure 2: Set-up for cell incubation with fresh cell culture medium. **Position A:** Empty. **Position B:** Dual reservoir adapter containing two 25 ml multichannel reagent reservoirs; one filled with 12 ml fresh cell culture medium (blue), and the other one to collect waste (magenta). **Position C:** INTEGRA slanted plate holder (20°) with the seeded cells in a 96 well flat bottom plate (green).



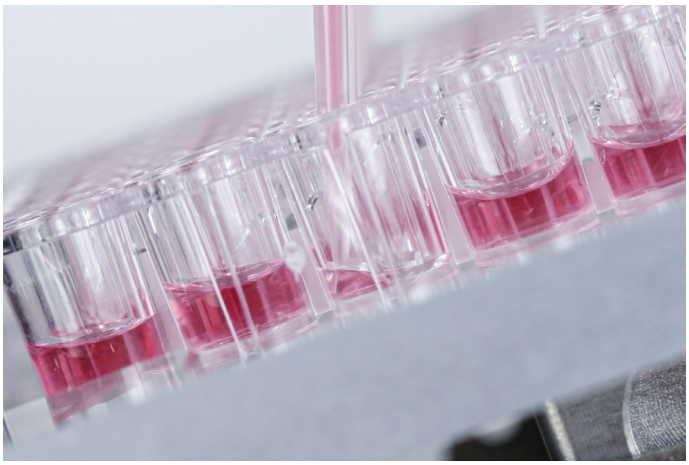


Figure 3: The INTEGRA slanted plate holder can tilt microplates at angles of 10°, 20° and 30°. This enables dispensing to the side wall of each well and complete aspiration of liquids, aiding gentle medium exchange to avoid detaching of cells.

Day 2 – Program 2 – Automated MTT assay

Experimental set-up

Deck position A: Dual reservoir adapter containing a 25 ml divided reagent reservoir and a 25 ml multichannel reagent reservoir with SureFlo anti-sealing array.
Deck position B: 150 ml automation friendly reagent reservoir with SureFlo anti-sealing array.
Deck position C: INTEGRA slanted plate holder (20°) with the seeded cells in a 96 well flat bottom plate (**Figure 4**, purple).

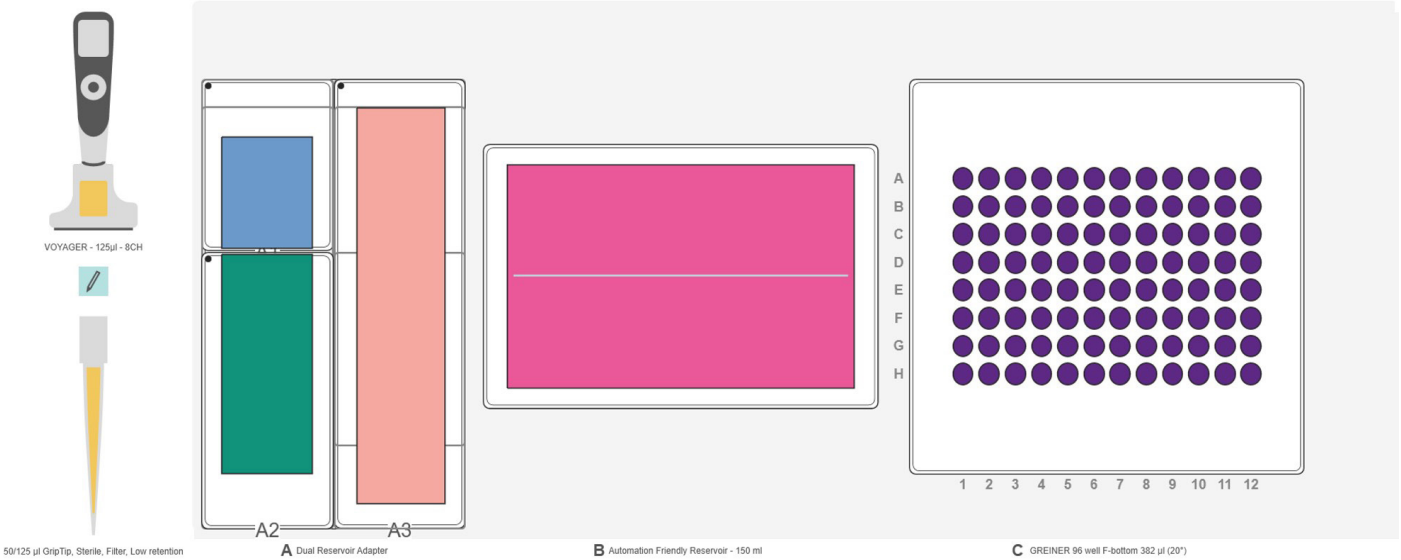


Figure 4: Deck set-up for the MTT assay. **Position A:** Dual reservoir adapter containing a 25 ml divided reagent reservoir filled with 1.5 ml MTT solution (blue) and 10 ml fresh cell culture medium (green), as well as a 25 ml reagent reservoir filled with 11 ml DMSO (light pink). **Position B:** 150 ml automation friendly reagent reservoir for the collection of the liquid waste (magenta). **Position C:** INTEGRA slanted plate holder (20°) with the seeded cells in a 96 well flat bottom plate (purple).

1. Cell washing	**STEP:** Washing the cells with fresh cell culture medium	**HOW TO:** Fill the 5 ml compartment of the divided reagent reservoir (blue) with 1.5 ml MTT stock solution (5 mg/ml) and the 10 ml compartment (green) with 10 ml of fresh cell culture medium. Place an empty 150 ml automation friendly reagent reservoir on deck position B to collect the liquid waste and the 96 well plate with the seeded cells on the slanted plate holder (20°) on deck position C (**Figure 4**). Select and run the VIALAB program 'MTT_assay' on the VOYAGER pipette. In this first step, the ASSIST PLUS pipetting robot exchanges the old cell culture medium for fresh medium. 100 µl of old medium is slowly aspirated from each well of the 96 well plate and replaced with fresh medium from the 10 ml compartment of the divided reagent reservoir. **Tips:** • The cell washing step can also be done with sterile PBS. • Disposable sterile filter tips allow for contamination-free cell culture processing. • Using Low Retention GripTips is optimal when pipetting liquids that contain proteins as they ensure the greatest liquid recovery in comparison with standard tips.
2. MTT addition and incubation	**STEP:** Adding MTT solution and incubating the cells to generate formazan crystals	**HOW TO:** The VOYAGER pipette adds 10 µl MTT solution to each well using Repeat Dispense mode. The ASSIST PLUS pipetting robot prompts the user to incubate the 96 well plate for 2-6 hours at 37 °C with 5 % CO_2. **Tips:** • The ASSIST PLUS pipetting robot can be paused at this step for the duration of the incubation or stopped. In the latter case, the VIALAB program 'MTT assay' should be continued from step 28 after the incubation. • Using the 25 ml divided reagent reservoir with SureFlo anti-sealing array allows a very low dead volume and minimizes the loss of expensive reagents.
3. Dissolving formazan crystals	**STEP:** Removing the cell culture medium containing residual MTT and dissolving the formazan crystals in DMSO	**HOW TO:** Fill the 25 ml reagent reservoir (**Figure 4**, light pink) with 11 ml DMSO. After incubation, when the purple formazan crystals are formed, the VOYAGER pipette carefully removes the cell culture medium containing residual MTT, slowly aspirating 110 µl from the 96 well plate and transferring it to the automation friendly reagent reservoir (**Figure 4**, magenta). Next, DMSO is added using the 'Repeat Dispense' mode and mixed thoroughly with the crystals. **Tips:** • During removal of the cell culture medium containing residual MTT, the aspiration speed is set to 2 to avoid disturbing the crystals. After the addition of DMSO, the mixing conditions (height, speed, cycle and volume) are optimized to allow complete dissolution. • Water-soluble tetrazolium dyes (e.g. XTT, MTS, WSTs) can also be used instead of MTT. In these cases, there is no need for dissolution in DMSO.

4. Incubation and plate reading	**STEP:** Incubation for complete dissolution of formazan crystals and results read-out	**HOW TO:** The ASSIST PLUS pipetting robot prompts the user to incubate the 96 well plate for 10 minutes at room temperature with 5 % CO_2. In the next step, the ASSIST PLUS pipetting robot alerts the user to read the results with a microplate reader at 570 nm (MTT) and 690 nm (for reference). **Tips:** • If dissolution of the formazan crystals is incomplete, incubate the plate overnight at 37 °C. • If a dye other than MTT is used, absorbance should be measured at the corresponding wavelength according to the supplier's recommendation.

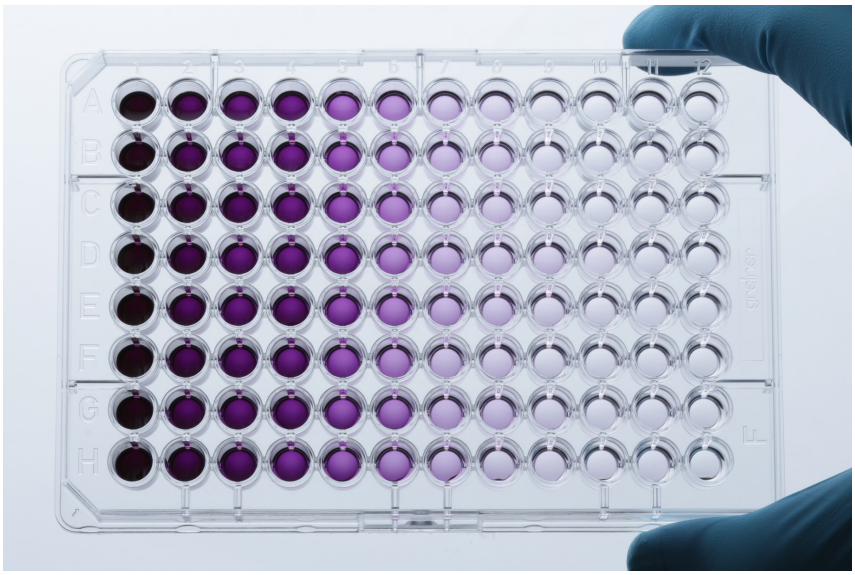


Figure 5: A 96 well plate after the MTT assay. Decreasing amounts of viable and metabolically active cells result in the decreasing intensity of the purple color observed.

Remarks

VIALAB software: The VIALAB program can easily be adapted to fit the user's specific labware and protocols.

Partial plates: Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

Conclusion

- Measurement of cell viability is widely used in estimating general cell health, assessing responses to toxic conditions, testing of drugs or screening active compounds. Conducting the MTT assay with the ASSIST PLUS pipetting robot offers a simple, fast, cost-effective and automated solution for assessing cellular metabolic activity.
- The small footprint of the affordable ASSIST PLUS pipetting robot means that it can easily be placed under a laminar flow cabinet, ensuring sterile working conditions.
- This assay is very flexible and can be easily adapted to user's needs. Changing the pipette allows the user to process plate formats other than 96 wells. Plates with 12-384 wells can also be processed on the ASSIST PLUS pipetting robot.
- Automated tip changes avoid any assay contamination, while using 'Repeat Dispense' and 'Multi Aspirate' modes whenever possible speeds up the process.
- Prolonged manual pipetting tasks lead to repetitive strain injury. This can be avoided by automating these steps with the ASSIST PLUS pipetting robot, maximizing hands-free time.

[1] T. Mosmann (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65 (16): 55-63.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/ en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	VOYAGER 8 channel 125 µl electronic pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4510	Slanted plate holder (0°-30°)	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	6565	125 µl Sterile, Filter, Low Retention GripTips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	4380 4381	25 ml Reagent Reservoir, Sterile, SureFlo anti-sealing array, polystyrene	https://www.integra-biosciences.com/switzerland/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6301 6302 6318	150 ml Automation Friendly Clear Advantage™ Reservoirs (Polystyrene)	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	4350 4351 4352	25 ml Divided Reagent Reservoir, Sterile, SureFlo anti-sealing array, polystyrene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/divided-reagent-reservoirs
Greiner Bio-One International	655161	96 Well Microplate, PS, F-Bottom	https://shop.gbo.com/en/germany/products/bioscience/microplates/96-well-microplates/96-well-microplates-clear/655161.html

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