
In His Name

History

Birjand Cellular and Molecular Research Center was founded in 2013 and aims at improving the fundamental and practical researches regarding the understanding of cellular and molecular basis of diseases. This center is ready to

train researchers in the fields of new technologies such as Biotechnology, Nanotechnology, Gene therapy, Cell therapy, etc. to reach the goals of Iran's Holistic Scientific Map.

Objectives

- Developing and using researchers' knowledge in cellular and molecular researches.
- Performing scientific researches (fundamental & practical) in order to expand the understanding and treating of diseases.
- Establishing data base regarding the cellular and molecular researches
- Attracting & using the actual and potential research talents.
- Trying to collaborate with other educational and research centers inside the country and abroad.
- Training researchers at MSc and Ph.D. programs related to the cellular and molecular fields.

Activities

⇒ Holding scientific events such as journal clubs and workshops to promote knowledge in the field of cellular and molecular research.

⇒ Collaborating tightly with Central Research Laboratory of Birjand University of Medical Sciences.

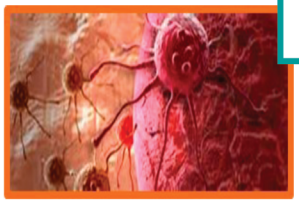
⇒ Collaborating with Bon-Yakhteh Institute

Research Priorities

The main focus of the research carried out by the research center is :

Molecular diagnosis and treatment of diseases

Bioinformatics Application in Medical Sciences



Prevention, diagnosis ,and treatment of cancer

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Chapter 1
Hoechst Staining

Hoechst Stains

Table 1. Contents and storage information.

Product	Amount	Concentration	Storage	Stability
Hoechst 33258, pentahydrate (bis-benzimide) FluoroPure™ grade (MW 623.96) (H21491)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33258, pentahydrate (bis-benzimide) (MW 623.96) (H1398)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33258, pentahydrate (bis-benzimide) (MW 623.96) (H3569)	10 mL	10 mg/mL (16.0 mM) solution in water	• Store at 2–6°C • Protect from light	When stored as directed, product is stable for 6 months.
Hoechst 33342, trihydrochloride, trihydrate, FluoroPure™ grade (MW 615.99) (H21492)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33342, trihydrochloride, trihydrate, (MW 615.99) (H1399)	100 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Hoechst 33342, trihydrochloride, trihydrate, (MW 615.99) (H3570)	10 mL	10 mg/mL (16.2 mM) solution in water	• Store at 2–6°C • Protect from light	When stored as directed, product is stable for 6 months.
Hoechst 34580, (MW 560.96) (H21486)	5 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
nuclear yellow (Hoechst S769121, trihydrochloride, trihydrate, (MW 651.01) (N21485)	10 mg	NA	• ≤25°C • Protect from light	When stored as directed, product is stable for at least 12 months.
Approximate Fluorescence Excitation and Emission, in nm: Excitation/Emission bound to DNA: Hoechst 33258 352/461 nm (see Figure 2); Hoechst 33342 350/461 nm; Hoechst 34580 392/440 nm (see Figure 3); Nuclear Yellow 355/495 nm.				

Introduction

The blue fluorescent Hoechst dyes (Figure 1) are cell permeable nucleic acid stains that have multiple applications, including sensitive detection (>3 ng) of DNA in the presence of RNA in agarose gels,[1] automated DNA determination,[2] sensitive determination of cell number [3,4] and chromosome sorting.[5] The fluorescence of these dyes is very sensitive to DNA conformation and chromatin state in cells. Consequently, they can detect gradations of nuclear damage. The Hoechst dyes are useful vital stains for the flow cytometric recognition of DNA damage [6,7] and other viability measurements by monitoring the emission spectral shifts of the dyes.[8] These bisbenzimidazole derivatives are supravital minor groove–binding DNA stains with AT selectivity.[9] The dyes

bind to all nucleic acids, but AT-rich dsDNA strands enhance fluorescence ~2-fold greater than GC-rich strands. This property has been used to identify Q-bands in chromosomes (Q-bands: AT-rich chromosome regions that fluoresce brightly when stained with the dye quinacrine).[10]

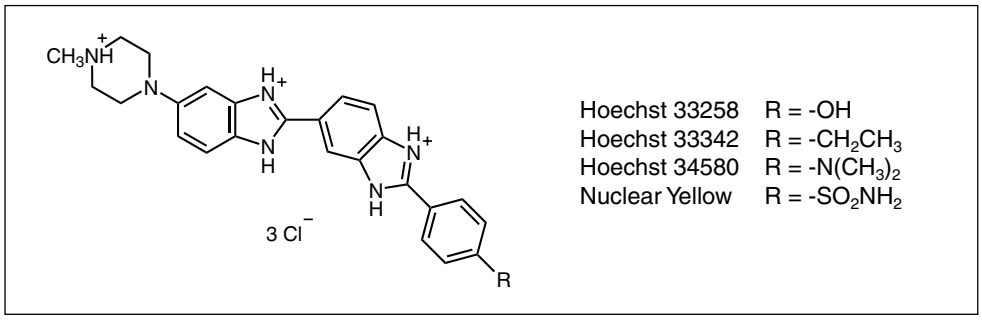

Figure 1. Structure of Hoechst dyes.

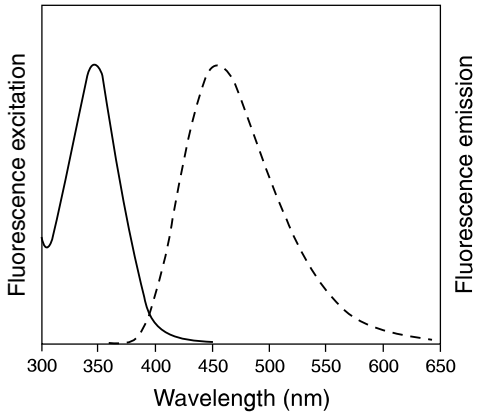

Figure 2. Fluorescence excitation and emission spectra of Hoechst 33258 bound to DNA

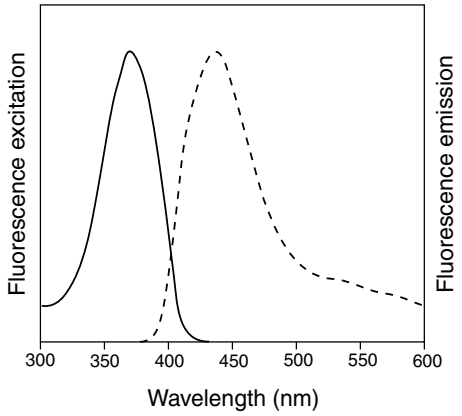

Figure 3. Fluorescence excitation and emission spectra of Hoechst 34580 bound to DNA.

BrdU Quenching

Hoechst 33342 and 33258 are quenched by BrdU.[11,12] Fluorescence is enhanced upon binding to dsDNA at stretches of at least three AT base pairs, but no binding to stretches of two or more GC base pairs. The Hoechst dyes requires a $[dA\text{-}dT]_3$-$[dG\text{-}dC]_1$ sequence to enhance fluorescence, with binding to the bottom of the minor groove as a prerequisite. It is postulated that the bromine on BrdU (or the chlorine on CldU) deforms the minor groove, such that the dye molecule can no longer reach its optimal binding site. It has shown that the Hoechst dyes still bind (with an even higher affinity) to BrdU substituted DNA, but no fluorescence ensues.[13] This has been used extensively to monitor cell cycle progression.

Hoechst 33258 is slightly more water soluble than Hoechst 33342, but both have been used extensively to stain live cells. The products may be used in fluorescence microscopy, microplate, cuvette, and flow cytometry applications. Nuclear yellow (Hoechst S769121) is more commonly used as a neuronal retrograde tracer.[14-16]

Preparing Stock Solutions of Hoechst Dyes

The solid dyes may be dissolved in either water, dimethylformamide (DMF), or DMSO to make concentrated stock solutions up to 10 mg/mL. Stock solutions may be stored refrigerated or frozen, protected from light. Note: The Hoechst stains should not be resolubilized in phosphate-buffered saline (PBS), but dilute solutions of the dye may be used with PBS or other phosphate-containing buffers. Solutions of Hoeschst dye should be stored at 2–6°C, protected from light. Stock solutions in water are stable for at least 6 months when refrigerated. For long-term storage the stock solution can be aliquoted and stored at ≤–20°C.

Caution

The Hoechst strains are known mutagens and should be handled with care. The dye must be disposed of safely and in accordance with applicable regulations.

Fluorescence Spectral Characteristics

The blue fluorescent Hoechst 33258, 33342, 34580 and nuclear yellow dyes may be efficiently excited with a xenon or mercury-arc lamp or with a UV laser; Hoechst 34580 may also be efficiently excited with non-UV wavelengths (>360 nm) such as the 405 nm laser line. They may all be detected using the common DAPI filter, blue GFP filters or the Semrock BrightLine® Alexa Fluor® 350 filter set.

Basic Protocol for Staining Cells

The following procedure can be adapted for most cell types. Note that different concentration ranges for the Hoechst dyes are suggested depending on the cell type (see Table 2). Growth medium, cell density, the presence of other cell types and other factors may influence staining. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present. Glassware should be washed in a mild detergent and rinsed with hot tap water followed by several rinses with deionized, distilled water.

Pellet cells by centrifugation and resuspend in buffered salt solutions or media, with optimal dye binding at pH 7.4. Adherent cells in culture may be stained *in situ* on coverslips. Add Hoechst stain using the concentrations listed in Table 2 as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining. Unbound dye has its maximum fluorescence emission in the 510–540 nm range, this green fluorescence may be observed on samples using too high a concentration of dye.

Table 2. Recommended conditions for staining cells with Hoechst stains.

Cell Type	Hoechst Dye Concentration	Incubation Conditions
Bacteria	0.1 to 12 µg/mL	10 to 30 minutes
Live animal cells	0.2 to 5 µg/mL	20 to 30 minutes
Fixed animal cells	0.2 to 2 µg/mL	1 to 15 minutes

References

1. Nucleic Acids Res 15, 10589 (1987); **2.** Anal Biochem 147, 462 (1985); **3.** Anal Biochem 131, 538 (1983); **4.** J Histochem Cytochem 29, 326 (1981); **5.** Cytometry 3, 145 (1982); **6.** Cytometry 11, 386 (1990); **7.** Cancer Res. 48, 5742 (1988); **8.** Cytometry 11, 239 (1990); **9.** BBA 949, 158 (1988); **10.** Chromosoma 46, 255 (1974); **11.** J Histochem Cytochem 24, 24 (1976); **12.** J Histochem Cytochem 25, 913 (1977); **13.** J Mol Biol 315, 1049 (2002); **14.** Neurosci Lett 18, 19 (1980); **15.** Exp Brain Res 40, 383 (1980); **16.** J Histochem Cytochem 30, 123 (1982).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
F2962	FluoReporter® Blue Fluorometric dsDNA Quantitation Kit *200-2000 assays*	1 kit
N21485	nuclear yellow (Hoechst S769121, trihydrochloride, trihydrate)	10 mg
H1398	Hoechst 33258, pentahydrate (bis-benzimide)	100 mg
H3569	Hoechst 33258, pentahydrate (bis-benzimide) *10 mg/mL solution in water*	10 mL
H21491	Hoechst 33258, pentahydrate (bis-benzimide) *FluoroPure™ grade*	100 mg
H1399	Hoechst 33342, trihydrochloride, trihydrate	100 mg
H3570	Hoechst 33342, trihydrochloride, trihydrate *10 mg/mL solution in water*	10 mL
H21492	Hoechst 33342, trihydrochloride, trihydrate *FluoroPure™ grade*	100 mg
H21486	Hoechst 34580	5 mg
V13244	Vybrant® Apoptosis Assay Kit #5 *Hoechst 33342/propidium iodide* *200 assays*	1 kit
V23201	Vybrant® Apoptosis Assay Kit #7 *Hoechst 33342/YO-PRO®-1/propidium iodide* *200 assays*	1 kit

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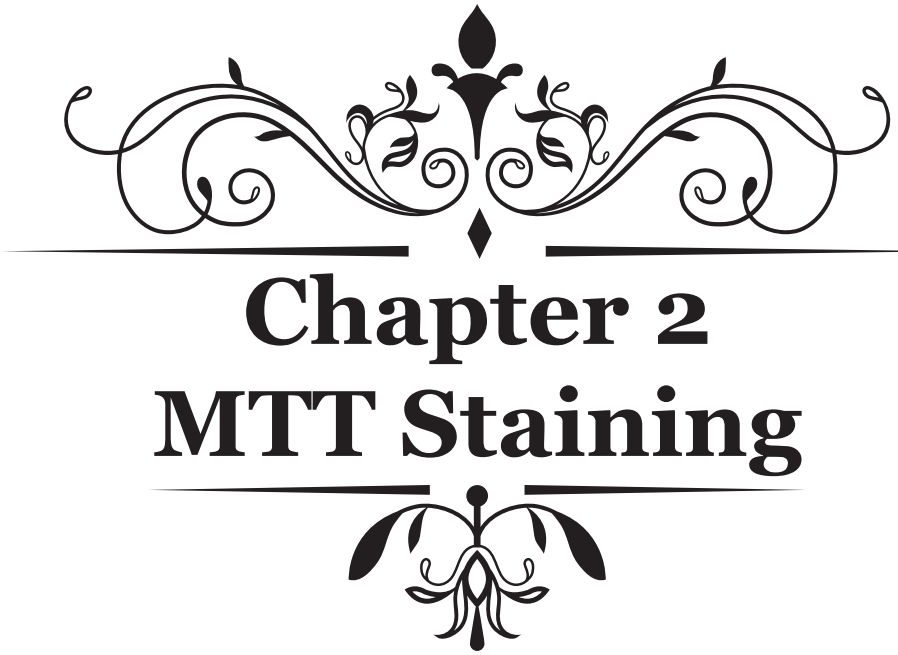
تست رنگ آمیزی آپوپتوز هوخست

یکی از روش هایی که به کمک آن می توان نوع مرگ سلولی را نشان داد، استفاده از رنگ هایی نظیر هوخست است که از رنگهای هسته دوست بوده و هسته سلول های آپوپتوزی و غیرآپوپتوزی را رنگ می کند. از آنجایی که سلول های آپوپتوزی دارای خصوصیات مورفولوژیک خاص، از جمله هسته قطعه قطعه و کروماتین متراکم می باشند، به کمک این نوع رنگ آمیزی به راحتی میتوان سلول های آپوپتوتیک را از سلول های غیرآپوپتوتیک تشخیص داد.

روش اجرا:

۱- کشت تعداد مناسب سلول (بسته به نوع سلول و پلیت مورد استفاده حداکثر تا ۱۰۰۰۰۰) و انکوباسیون به مدت ۲۴ ساعت در ۳۷ درجه
۲- تیمار با ماده مورد نظر و انکوباسیون در ۳۷ درجه به مدت ۲۴، ۴۸ و ۷۲ ساعت
۳- جمع آوری مدیای رویی حاوی سلول های مرده در میکروتیوب ۱,۵
۴- تریپسینه کردن سلول های چسبیده به چاهک ها و اضافه کردن آن ها به میکروتیوب های قبلی
۵- سانتریفیوژ کردن میکروتیوب ها به مدت ۳ دقیقه در ۱۵۰۰ RPM
۶- خارج کردن سوپرناتانت
۷- حل کردن رسوب سلولی در ۱۰۰ میکرولیتر از محلول ۱۰٪ فرمالین حاوی Hoechst 33258 dye 25/6 ng/ml و انکوباسیون به مدت ۲۴ ساعت در تاریکی و در ۴ درجه
۸- پس از ۲۴ ساعت، کاملا تصادفی یک میکروتیوب را برداشته و ۱۰ میکرولیتر از محتویات آن با پیپتاژ جدا کرده و روی لام قرار می دهیم و سپس با بزرگنمایی ۴۰ و با نور لیزر با میکروسکوپ فلوئورسنت تغییرات مورفولوژیک هسته سلول ها را حداقل در ۲۰۰ سلول بررسی می کنیم و درصد سلول های آپوپتوتیک را محاسبه می کنیم.



Chapter 2
MTT Staining

INTRODUCTION

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means.

The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

KIT COMPONENTS

Component	Volume	Storage
MTT Reagent	25 mL	4°C
Detergent Reagent	2 × 125 mL	Room Temp. or 4°C

The MTT Reagent is ready to use and stable at 4°C in the dark for up to eighteen months, provided there is no contamination. Care should be taken not to contaminate the MTT Reagent with cell culture medium during pipetting. We recommend that the appropriate volume required for each experiment be removed and aseptically placed into a separate clean tube and the stock bottle returned to 4°C in the dark. If the MTT Reagent is bluegreen, do not use and refer to the troubleshooting guide on page 6.

The Detergent Reagent is supplied ready to use. If the Detergent Reagent has been stored at 4°C, warm the bottle for 5 minutes at 37°C then mix by inverting gently to avoid frothing. The detergent is stable for up to eighteen months at room temperature.

EQUIPMENT AND MATERIALS REQUIRED BUT *NOT* SUPPLIED

Microtiter plate reader with 650- and 570-nm filters	Microtiter plate (flat-bottomed)
Inverted microscope	Sterile tubes (5 mL)
Multi-channel pipette	Serological pipettes
37°C incubator	Sterile pipette tips
Laminar flow hood	

BASIC PROTOCOL

If you are familiar with the procedure and know the cell count to use in your specific assay, you may follow this basic protocol.

Step	Action
1	Plate cells at 1,000 to 100,000 per well.
2	Incubate for 6 to 24 hours.
3	Add 10 µL MTT Reagent.
4	Incubate for 2 to 4 hours until purple precipitate is visible.
5	Add 100 µL Detergent Reagent.
6	Leave at room temperature in the dark for 2 hours.
7	Record absorbance at 570 nm.

DETERMINING OPTIMAL CELL COUNTS

Use the protocol below to determine the optimal cell count and incubation period for your cell line. This determination should only have to be done once for each cell type. The data will be used thereafter in your experimental system following the protocol above.

Step	Action
1	Harvest suspension cells by centrifugation. Adherent cells should be released from their substrate by trypsinization or scraping.
2	Resuspend cells at 1 x 10^6 per mL.
3	Prepare serial dilutions of cells in culture medium from 1 x 10^6 to 1 x 10^3 cells per mL.
4	Plate out, in triplicate, 100 µL of the dilutions into wells of a microtiter plate.
5	Include three control wells of medium alone to provide the blanks for absorbance readings.
6	Incubate the cells under conditions appropriate for the cell line for 6 to 48 hours (to recover from handling). The time required will vary but 12 hours to overnight is sufficient for most cell types.
7	Add 10 µL of MTT Reagent to each well, including controls.
8	Return plate to cell culture incubator for 2 to 4 hours.
9	Periodically view the cells under an inverted microscope for presence of intracellular punctate purple precipitate.
10	When the purple precipitate is clearly visible under the microscope add 100 µL of Detergent Reagent to all wells, including controls. Swirl gently; do not shake.
11	Leave plate with cover in the dark for 2 to 4 hours or overnight at room temperature.
12	Remove plate cover and measure the absorbance in each well, including the blanks, at 570 nm in a microtiter plate reader. [Absorbances can be read with any filter in the wavelength range of 550 - 600 nm. The reference wavelength should be higher than 650 nm. The blanks should give values close to zero (+/- 0.1).]
13	If the readings are low return the plate to the dark for longer incubation.
14	Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance against number of cells/mL. The number of cells to use in your assay should lie within the linear portion of the plot and yield an absorbance of 0.75 - 1.25.

PERFORMING AN ASSAY

The plot of the data obtained in Step 14 on page 3 (absorbance against number of cells) should provide a curve with a linear portion. The optimal number of cells for the assay should fall within the linear portion of the curve and give an absorbance value between 0.75 and 1.25. Then both stimulation and inhibition of cell proliferation can be measured.

To run an assay, select an optimal cell number and follow the MTT Cell Proliferation Assay steps 4 to 13 (page 3) using your experimental system, plating in triplicate. Assays will include:

a) Blank wells containing medium only
b) Untreated control cells
c) Test cells treated with the substance to be assayed

If more than 100 µL of medium is used per well, increase the amount of MTT Reagent accordingly; e.g., for 250 µL of medium use 25 µL of MTT Reagent.

DATA INTERPRETATION

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

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Mosmann, T. J. Immunol. Methods 65: 55-63, 1983.

TROUBLESHOOTING

Problem: MTT Reagent is blue-green.

Cause	Remedy
Contamination with a reducing agent or cell/ bacterial contamination.	Discard. Remove aliquots of new MTT Reagent using sterile technique.
Excessive exposure to light.	Store solution in the dark at 4°C.

TROUBLESHOOTING (CONTINUED)

Problem: Blanks (medium only) give high absorbance readings.

Cause	Remedy
The medium is contaminated with cells/ bacteria/yeast (visible under microscope).	Discard. Check medium before plating. Use sterile technique for cell plating in biological hood. Use sterile 96-well plate.
The medium contains ascorbic acid.	Incubate plate in the dark. Find alternative medium if possible.

Problem: Absorbance readings too high.

Cause	Remedy
Cell number per well too high.	Decrease cell density at plating.
Contamination of culture with bacteria or yeast.	Discard. View wells prior to addition of MTT Reagent to check for contamination.

Problem: Absorbance readings are too low.

Cause	Remedy
Cell number per well is too low.	Increase cell density at plating.
Incubation time for reduction of MTT is too short. No purple color visible in cells when viewed under microscope.	Increase incubation time with MTT Reagent until purple color is evident inside cells when viewed under microscope. Longer incubation of up to 24 hours may be required for some cell types.
Incubation time for solubilization of formazan dye too short (intact cells with intracellular dye visible when viewed under the microscope).	Increase incubation time with Detergent Reagent or incubate at 37°C. View under microscope to ensure no crystals remain out of solution.
Cells not proliferating due to improper culture conditions or inadequate time of recovery after plating.	Check that culture conditions (medium, temperature, humidity, CO_2, etc.) are appropriate. View cells periodically to check condition. Increase time in culture after plating for cell recovery.

Problem: Replicates have different values.

Cause	Remedy
Inaccurate plating or pipetting.	Increase accuracy of cell plating, check accuracy of pipette.

SAFETY

See the Material Safety Data Sheet regarding safety precautions for this product.

RELATED PRODUCTS

ATCC has the world's largest collection of cell lines. If you need a cell line as a control for performing your assay, see our Web site at www.atcc.org to search our online catalog. We also offer cell culture media, serum, and reagents.

تست MTT جهت بررسی قابلیت حیات سلول ها

اساس روشMTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) بر پایه احیای سوبسترای زرد رنگ تترازولیوم به واسطه آنزیم های میتوکندریایی دهیدروژناز در سلول های زنده و تبدیل آن به ترکیب بنفش رنگ فورمازان است. شدت رنگ تولید شده پس از حل نمودن رسوب فورمازان در حلال های آلی نظیر دی متیل سولفوکسید (DMSO) با استفاده از روش اسپکتروفوتومتری قابل اندازه گیری است و رابطه مستقیمی با تعداد سلو ل های زنده دارد.

روش اجرا:

۱- کشت تعداد مناسب سلول (بسته به نوع رده سلولی مورد مطالعه حدود ۵۰۰۰-۸۰۰۰ سلول) در پلیت ۹۶ خانه و انکوباسیون در ۳۷ درجه به مدت ۲۴ ساعت

۲- آسپیراسیون مدیای رویی

۳- تیمار سلول ها با ماده مورد نظر:

به این منظور ماده مورد مطالعه در مدیای بدون FBS و در صورت لزوم ۱٪ DMSO حل شده و با غلظت مناسب به چاهک ها اضافه می شود. سپس حجم مدیای چاهک ها با مدیای بدون FBS به ۲۰۰ می رسد و پلیت برای بازه زمانی مدنظر (۲۴،۴۸ و ۷۲ ساعت) در انکوباتور ۳۷ درجه قرار می گیرد.

۴- در هر یک از زمان های مورد مطالعه برای بررسی میزان مرگ سلولی به این صورت عمل می شود:

آسپیراسیون مدیای رویی

شستشو با PBS

اضافه کردن ۲۰۰ میکرو لیتر محلول MTT با غلظت ۰,۵ mg/ml به هر چاهک

انکوباسیون به مدت ۳- ۴ ساعت در تاریکی و در ۳۷ درجه

آسپیراسیون محلول MTT

۵- در آخرین زمان مدنظر برای مطالعه پس از آسپیراسیون MTT به این صورت عمل می کنیم:

اضافه کردن ۱۵۰ میکرولیتر DMSO به هر چاهک و حل کردن کامل رسوب ایجاد شده

خوانش در طول موج های ۵۷۰ و ۶۲۰ (طول موج رفرنس)

درصد سلول های زنده با استفاده از فرمول زیر محاسبه می شود:

Cell viability% =(OD treated group/OD untreated group)*100

IC ۵۰ پس از رسم منحنی با بکارگیری غلظت‌های مختلف و درصد سلول های زنده محاسبه می شود. غلظتی که تعداد سلول های زنده را بعد از هر زمان پنجاه درصد کاهش دهد به عنوان IC ۵۰ در نظر گرفته می شود.