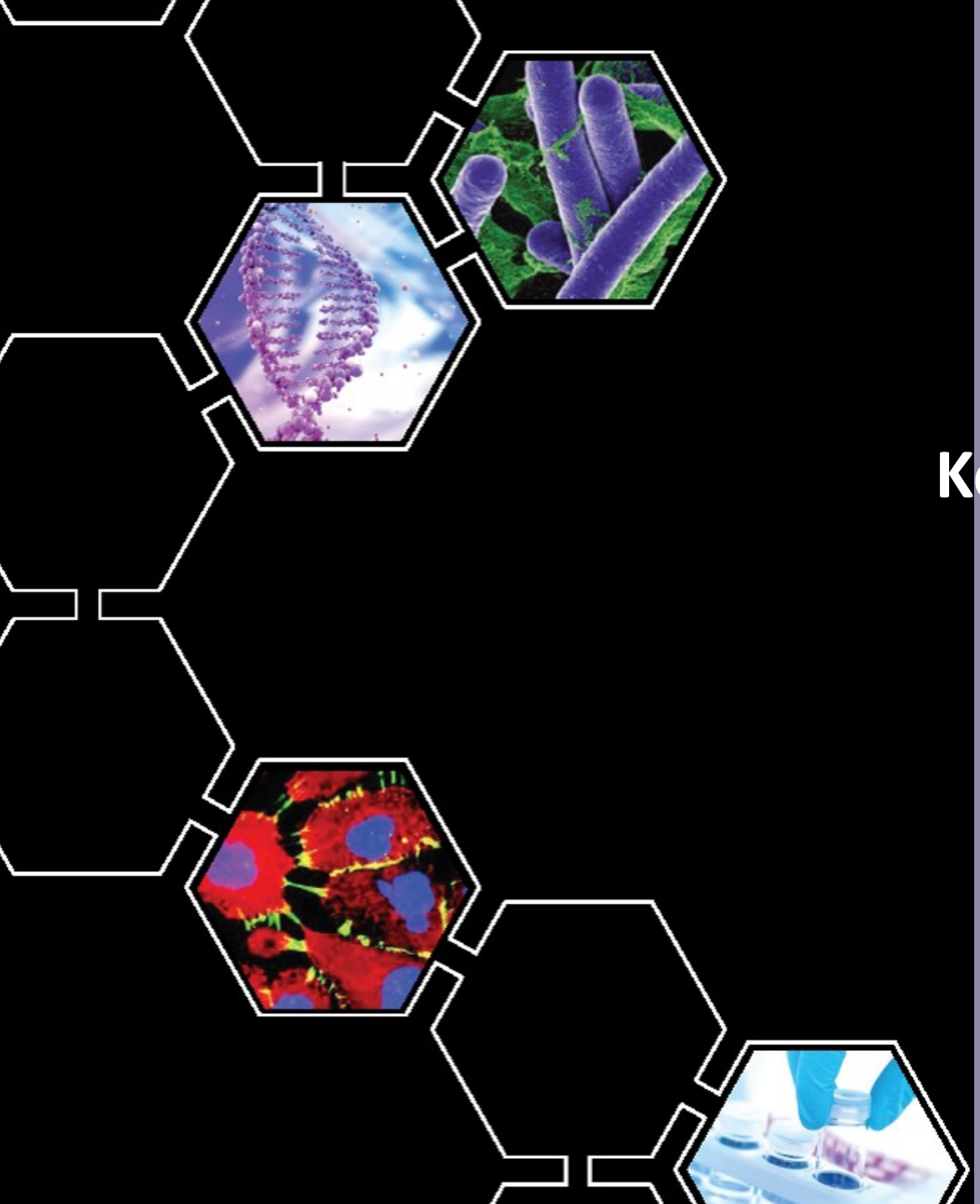


Keeping Cells Happy – Topics in Cell Health Maintenance and Viability

Steve Budd, M.S.

Product Line Business Specialist, ATCC

ATCC Overview

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA and an R&D & Services center in Gaithersburg, MD
- World wide brand name and quality recognition

- World's premiere biological materials resource and standards development organization
  - 4,000 cell lines
  - 70,000 microbes

- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in 140 countries, 12 International distributors

- Talented team of 475+ employees; > one third with advanced degrees

- Multiple accreditations including ISO 9001 and ISO 13485

Established partner to global researchers

Outline

Cell Health and Viability Topics

- Cell Culture
  - Media
  - Additives / Serum
- Cryopreservation / Post Thaw
- Cell Proliferation / Viability
  - MTT / XTT kits
  - Reliablue™ cell viability reagent
- Mycoplasma Effects / Detection

Complete growth media

- Classical cell culture media
- Media ingredients
- Additives
- Animal sera

Media choices

Animal cell lines – media + 10% FBS

- Eagle's Minimum Essential Medium (EMEM; ATCC® 30-2003™)
- Dulbecco's Modified Eagle's Medium (DMEM; ATCC® 30-2002™)
- Iscove's Modified Dulbecco's Medium (IMDM; ATCC® 30-2005™)
- Kaighn's Modification of Ham's F-12 Medium (ATCC® 30-2004™)
- DMEM/ F12 Medium (ATCC® 30-2006™)
- McCoy's 5A (ATCC® 30-2007™)
- RPMI-1640 (ATCC® 30-2001™)
- Leibovitz's L-15 (ATCC® 30-2008™)

Primary Cells – Primary Cell Basal Media and Growth Kits

- Primary cells require their own specially formulated media, specific to each cell type

Media ingredients

Sodium bicarbonate

$H_2O + CO_2 \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$

$H_2O + CO_2 \longleftrightarrow NaHCO_3 \longleftrightarrow H^+ + 2HCO_3^-$

HEPES buffer

- Can buffer without CO_2 enrichment
- Good for working under the hood

Phenol Red

- Monitors pH of media
- Yellow = acidic
- Purple = basic
- May mimic action of steroid hormones.

Sodium Pyruvate

- Helps maintain metabolism

Additives

Nonessential Amino Acids

- Can be added to reduce the metabolic burden on cells

L-Glutamine (ATCC® 30-2214™)

- Present in ATCC classical cell culture media
- Relatively stable in bottles kept at 4°C - 8°C
- ***Glutamine degradation increases ammonia toxicity***
- ***Generally not recommended to "spike" media with L-Glutamine***

Antibiotics and Antimycotics

- Penicillin-Streptomycin, Gentamicin Sulfate
- Amphothericin B
- ***Generally not recommended***

Animal sera

Fetal Bovine Serum (ATCC® 30-2020™)

Fetal Bovine Serum, Embryonic Stem Cell Qualified (ATCC® SCRR-30-2020™)

- Very rich in growth factors, most common choice
- **Heat inactivation: Not Advised**

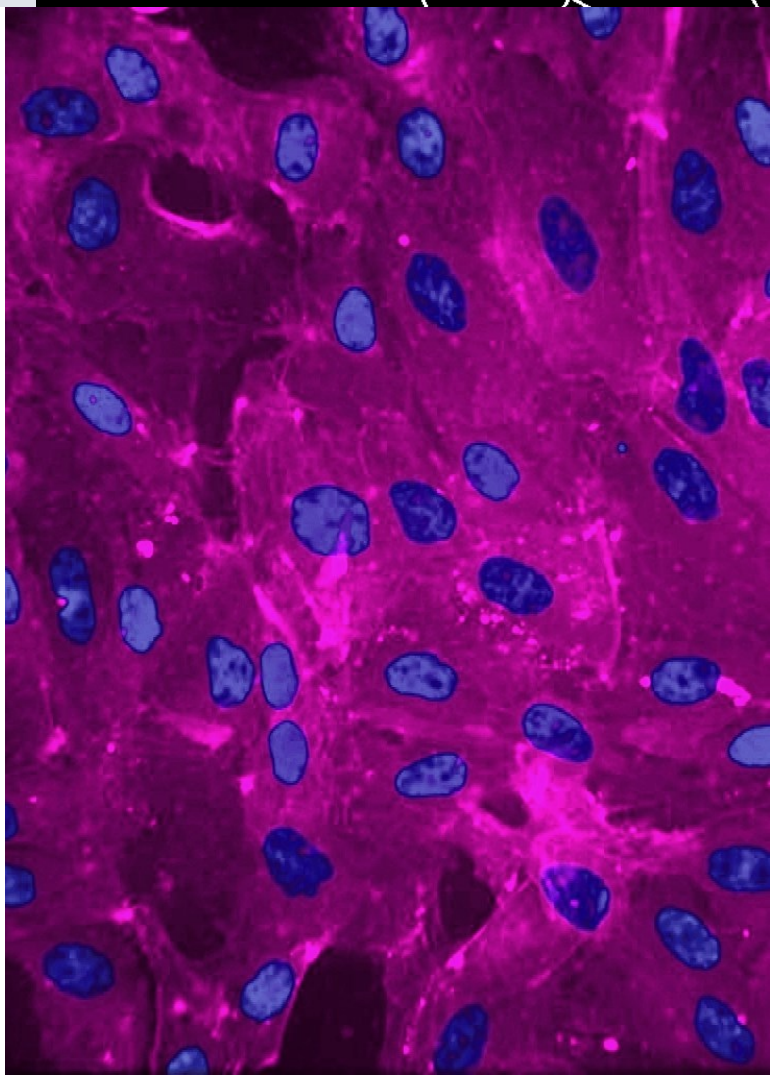
Calf Bovine Serum (ATCC® 30-2030™)

- Lower concentrations of growth factors, good for contact inhibition studies

Horse Serum (ATCC® 30-2040™)

- Collected from closed herds, lot-to-lot consistency, no bovine viruses

Media usage considerations



Maintain cells in the same media

Media variability

- Possible osmotic shock

When transferring to new media:

- Use 1:1 mix (50% old, 50% new media)
- 1:2 mix
- 1:3 mix
- 1:7 mix

ATCC® Animal Cell Culture Guide

Outline

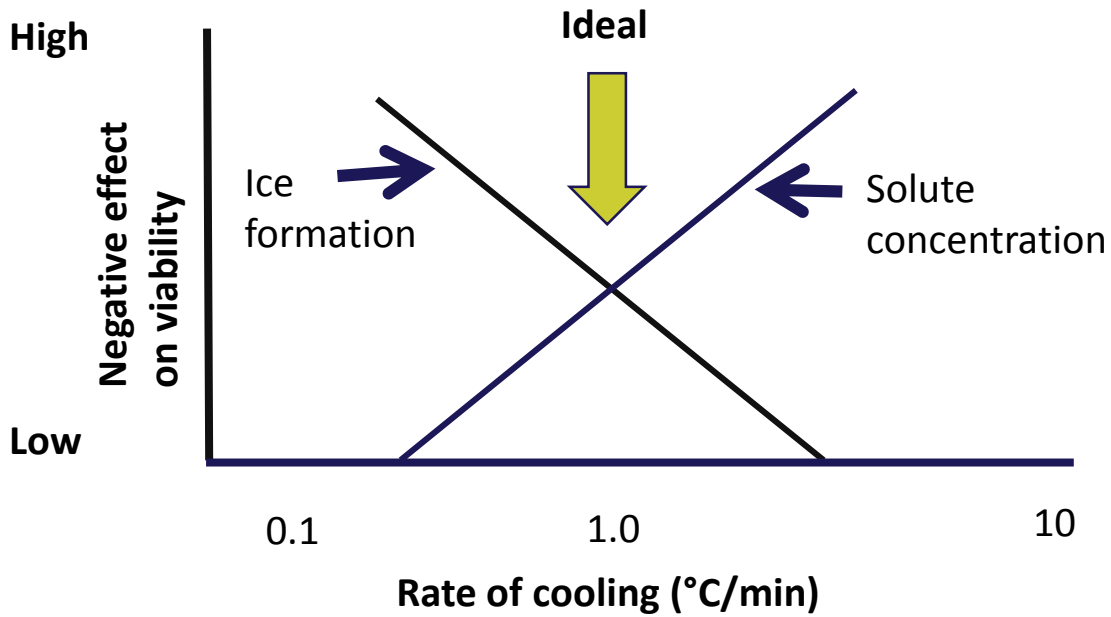
Cell Health and Viability Topics

- Cell Culture
  - Media
  - Additives / Serum
- **Cryopreservation / Post Thaw**
- Cell Proliferation / Viability
  - MTT / XTT kits
  - Reliablue™ cell viability reagent
- Mycoplasma Effects / Detection

Cryopreservation procedure

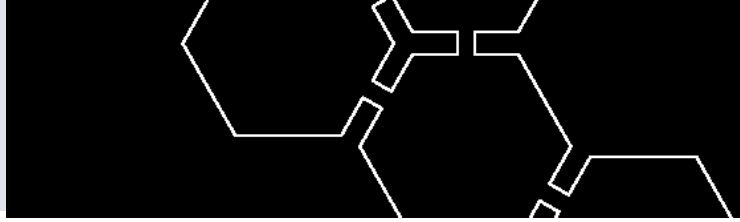
- Overview
- Cryoprotectants and media preparation
- Freezing cells in a controlled-rate chamber
- Long-term storage

Cryopreservation principles



- High levels of ice formation and increased solute concentration have a negative impact on cell viability
- Optimal cooling rate for cell viability is 1°C/min to 3°C/min

Cryoprotectants



Cell type	Cryoprotectant	Temperature
Animal cells	DMSO (5-10%) or Glycerol (5-10%)	-140°C
Bacteria	Glycerol (5-10%)	-80°C
Yeast	Glycerol (10%)	-140°C
Protozoa	DMSO (5-10%) or Glycerol (10-20%)	-140°C
Plant cells	DMSO (5-10%) and Glycerol (5-10%)	-140°C
Animal viruses (free)	None	-80°C
Animal viruses (infected cells)	DMSO (7%)	-10°C

Thawing cells

- Thaw in 37°C water bath for approximately 2 minutes with gentle agitation
- Spray vial with 70% ethanol
- Transfer to 10 mL centrifuge tube with 9 mL of appropriate growth media (10% FBS)
- *Centrifuge, resuspend in 2 mL of growth media
- Transfer to cell culture vessel

When bringing out of liquid nitrogen, thaw as quickly as possible

***For certain primary cells, centrifugation may be detrimental, refer to specific protocol**

Freezing Media preparation

Classical cell culture media – DMEM, EMEM, RPMI-1640 (for suspension cells)

- 5-10% DMSO
- 20% fetal bovine serum (FBS) or bovine serum albumin (BSA)
  - Additional cryprotectant properties
  - Necessary for post-thaw cell survival

ATCC Serum-free Freezing Media (ATCC® 30-2600™)

- All in one media
- 10% DMSO with proteins and additives for cell survival

Freezing cells

-70°C

Controlled rate freeze chamber

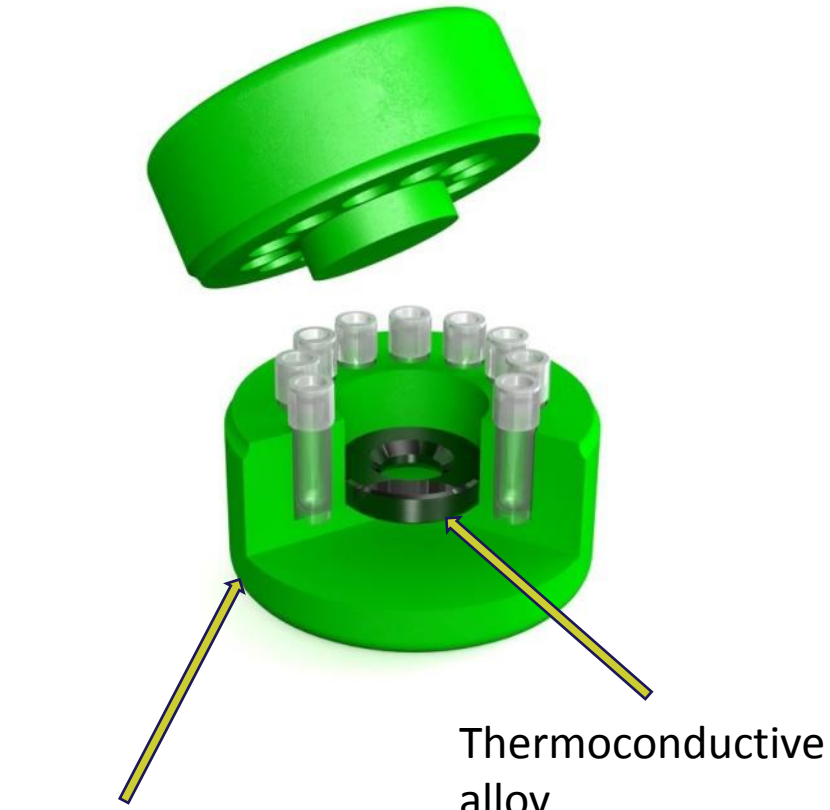
-1°C/min cooling rate

A few hours to 24 hours

-140°C

Liquid nitrogen tank

Freezing cells



CoolCell® LX (ATCC® ACS-6000™)

- Reliable -1°C/min cooling rate
- 4 hours in -70°C freezer
- Comfortable to touch
- No alcohol use or maintenance
- Can be used with all cell types
  - Verified use with organoids

Low temperature storage

For the best security, always store your cells in liquid nitrogen freezers

Low temperature storage

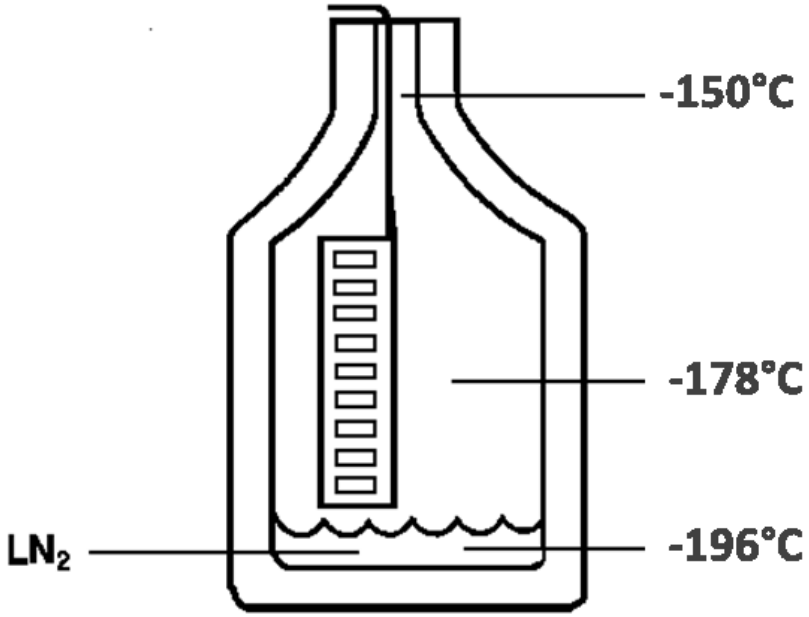
Mammalian cells

Long-term storage should be below -140°C

- -140°C for an indefinite length of time
- -80°C for less than 1 year

Vials should be stored in a liquid nitrogen unit ***above*** the volume of liquid, in the vapor phase

This temperature should be between **-140°C** and **-180°C**



Outline

Cell Health and Viability Topics

- Cell Culture
  - Media
  - Additives / Serum
- Cryopreservation / Post Thaw
- **Cell Proliferation / Viability**
  - **MTT / XTT kits**
  - **Reliablue™ cell viability reagent**
- Mycoplasma Effects / Detection

Population doubling level

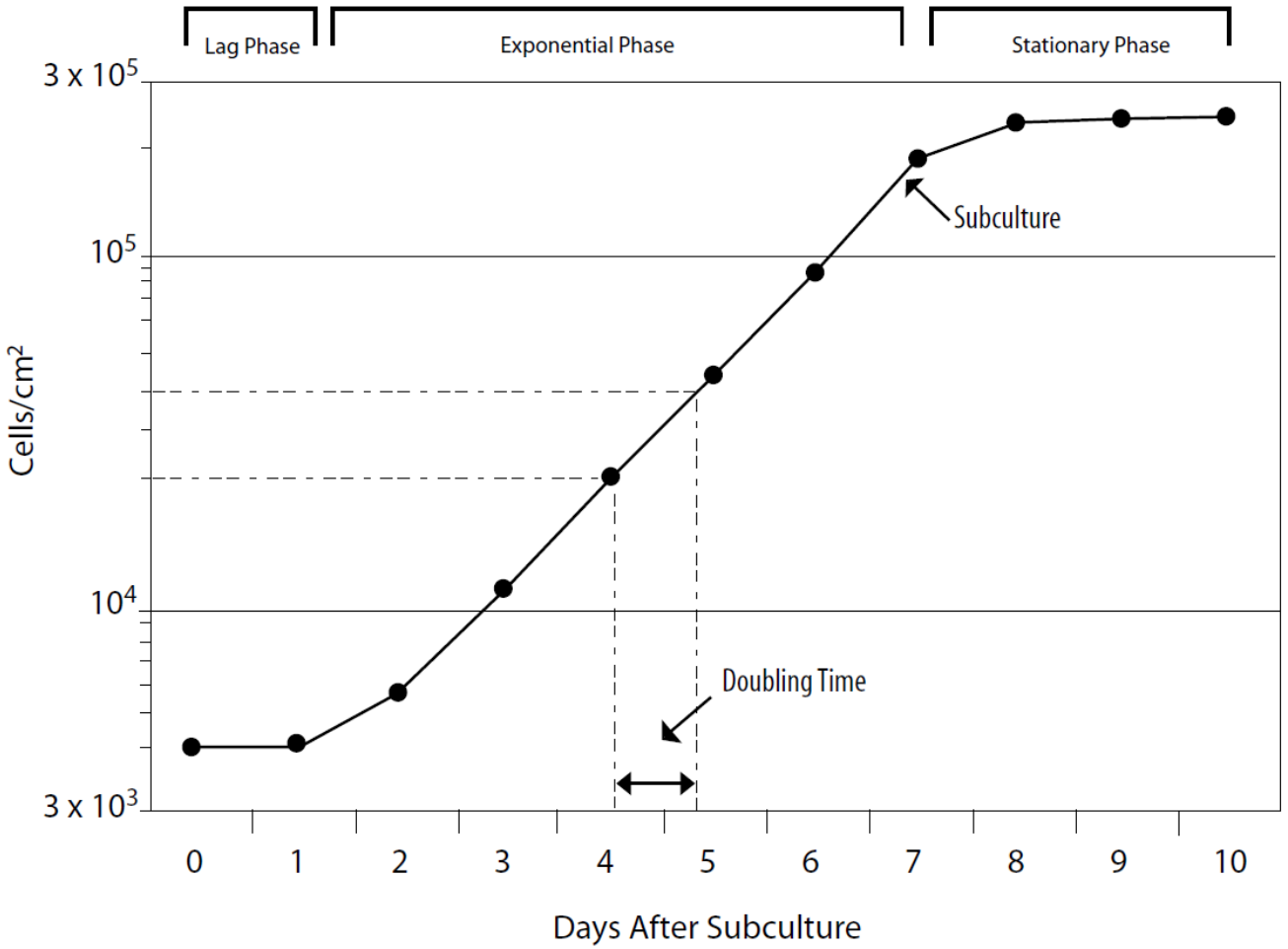
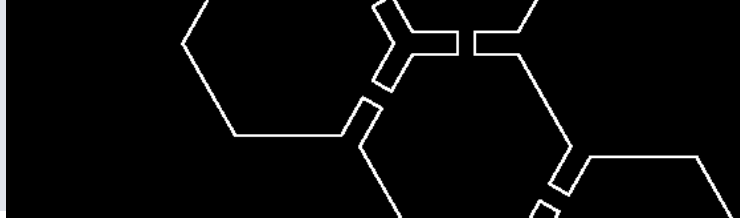


Figure 1. Growth curve for cells grown in culture. Cells should be subcultured while still in the exponential phase.

Growth and viability



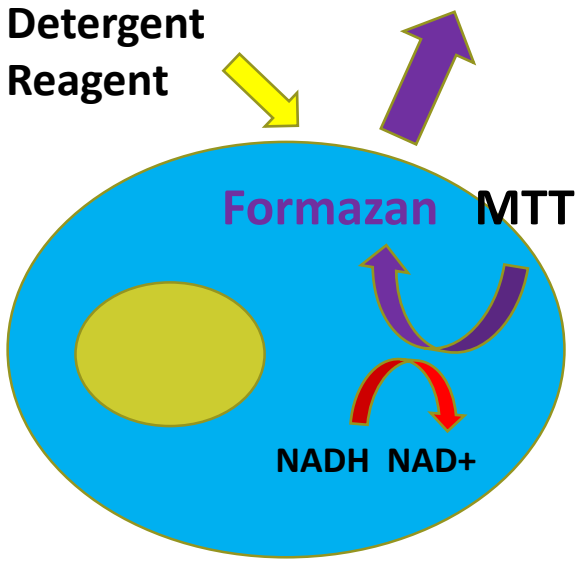
- Quantitative evaluation of **cell proliferation rate** and response to external factors that affect cell viability
- Uses tetrazolium salts in a colorimetric method for evaluating cell populations

- **MTT Cell Proliferation Assay (ATCC® 30-1010K™)**
  - Tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)
- **XTT Cell Proliferation Assay (ATCC® 30- 1011K™)**
  - Tetrazolium XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium)

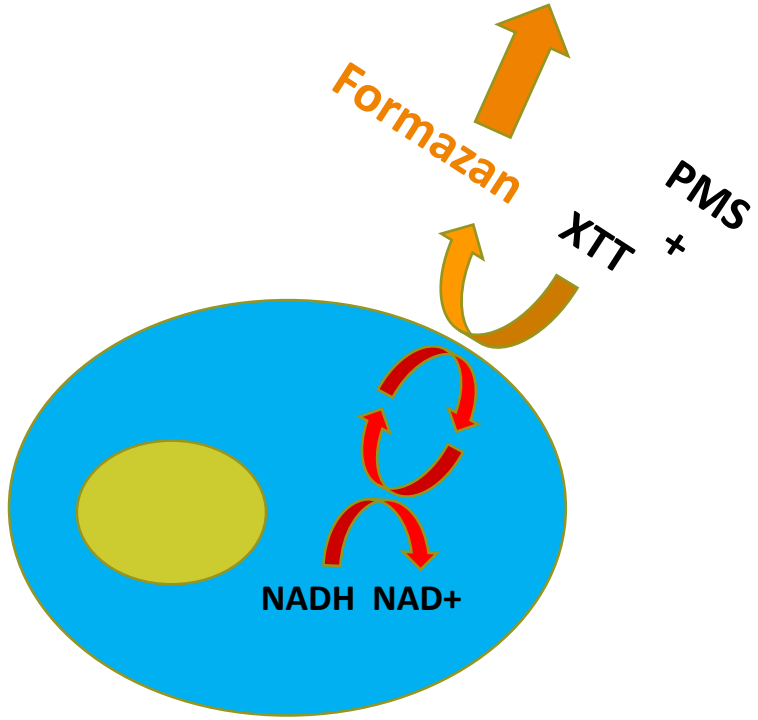
- **Reliablue™ Cell Proliferation Reagent (ATCC® 30-1014)** New!
  - Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide,

MTT / XTT

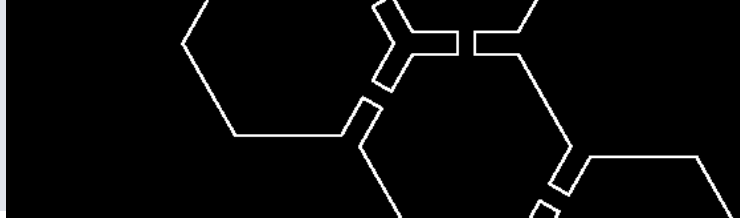
- **MTT Reaction**
- MTT salt is **reduced** within cellular matrix to Formazan, lysed with detergent to solubilize crystals
- Media turns **PURPLE**



- **XTT Reaction**
- XTT salt is **reduced** to Formazan at cell membrane with PMS agent
- Media turns **ORANGE**



MTT / XTT



Determining Optimal Cell Counts

- Plate, in triplicate, a serial dilution of 1 x 10^6 to 1 x 10^3 cells per mL (96-well plate)

MTT Assay

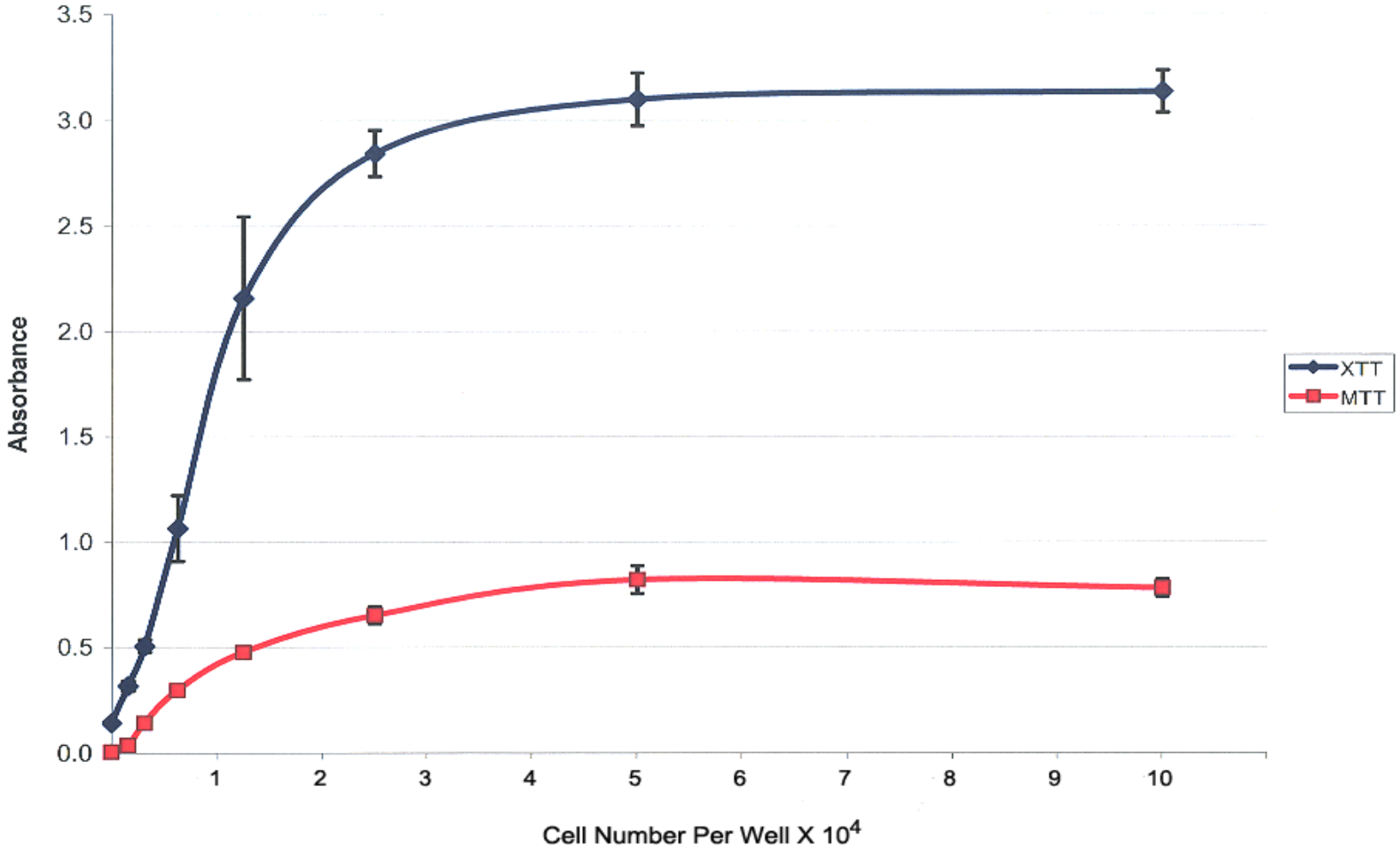
- Add MTT Reagent
- Incubate 2-4 hours – add Detergent
- Incubate 2-4 hours or overnight

XTT Assay

- Add XTT Reagent + Activation Agent
- Incubate 2-4 hours

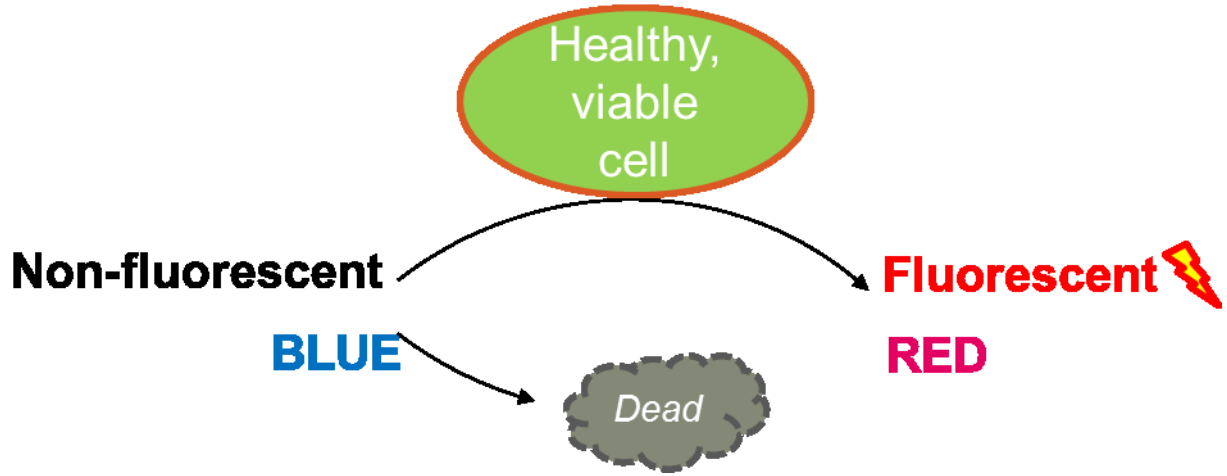
- Determine optimal number of cells to use
- Repeat assay with experimental factors – compare absorbance at optimal cell volume
- Plots absorbance versus cell number

Growth curves

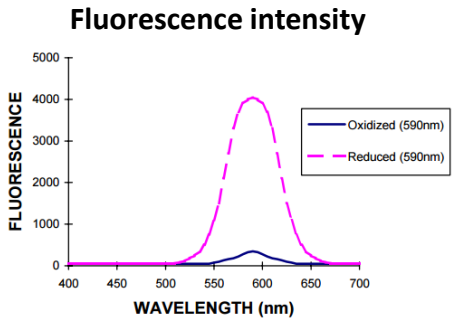


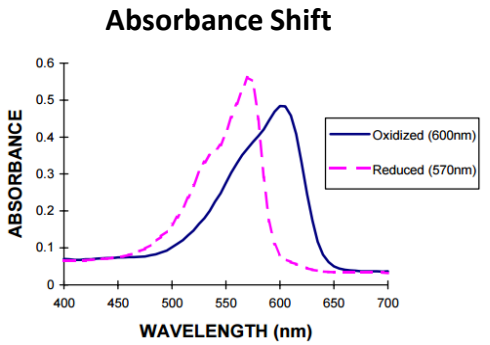
Reliablue™ Cell Viability Reagent

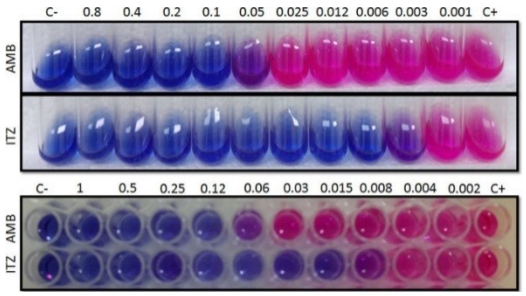
Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue dye that is weakly fluorescent until reduced (redox) at which point it becomes pink and highly fluorescent.



Resazurin is cell permeable but non-toxic and is metabolically reduced by living cells (but not dead cells or in the culture media) resulting in a change in absorbance and increase in fluorescence.



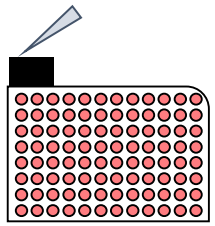




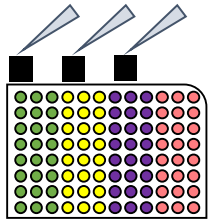
Reliablue™ Cell Viability Reagent

Reliablue™ Reagent is supplied in a 10X ready-to-use format that can be added directly to cells, typically in multiwell plates. An overview of the workflow is shown below.

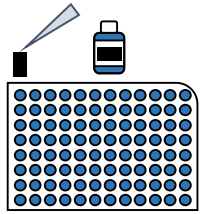
Basic 4-Step Assay Workflow



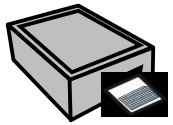
① Grow cells



② Add treatments



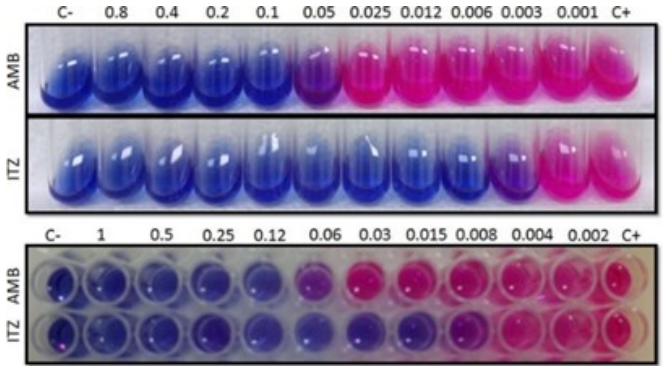
③ Add Reliablue



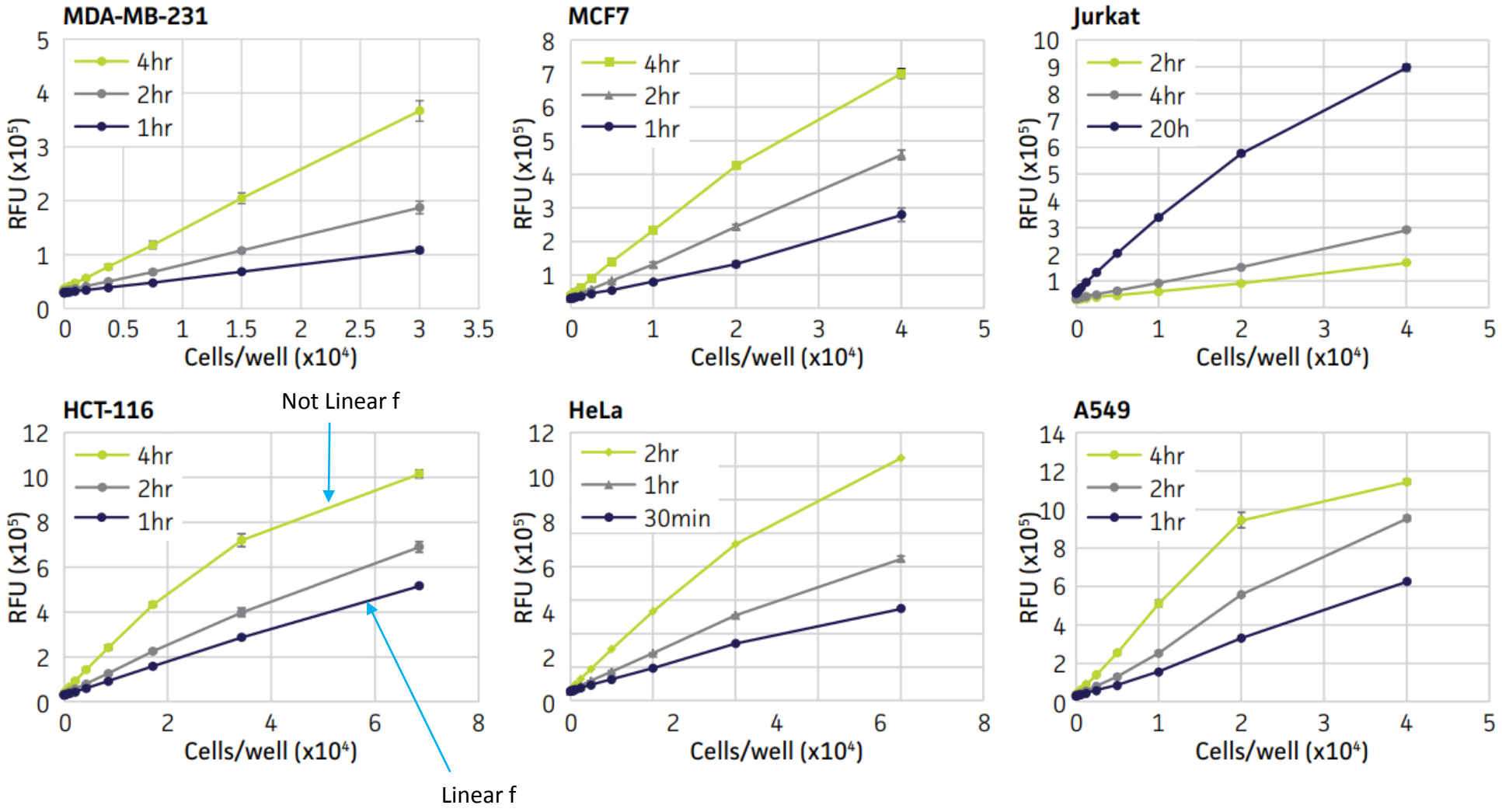
④ Read on plate-reader

Blue = Dead (or control)

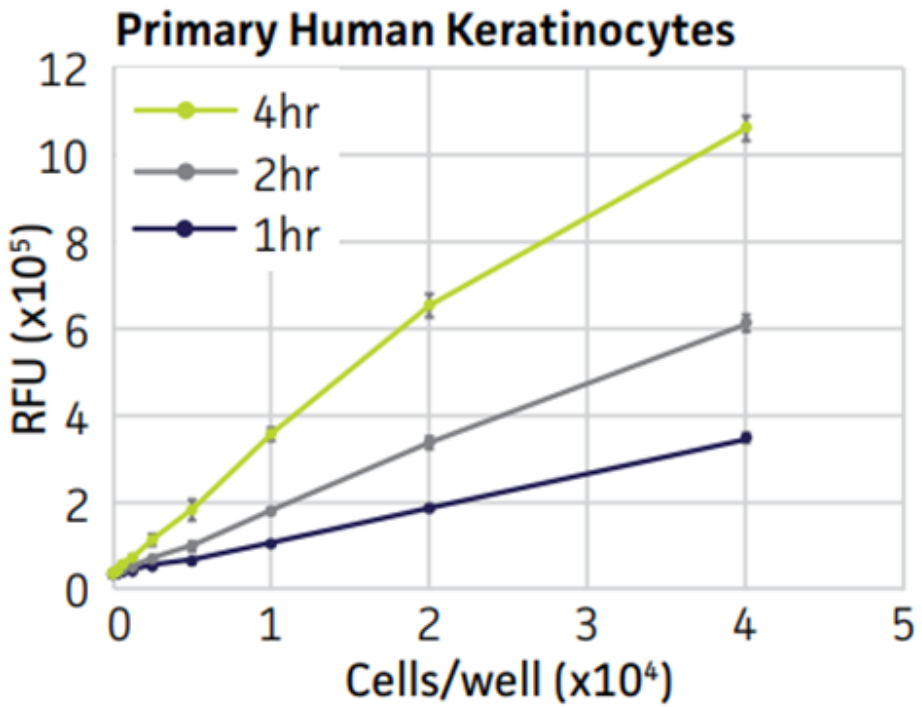
Red = Alive and Actively Growing

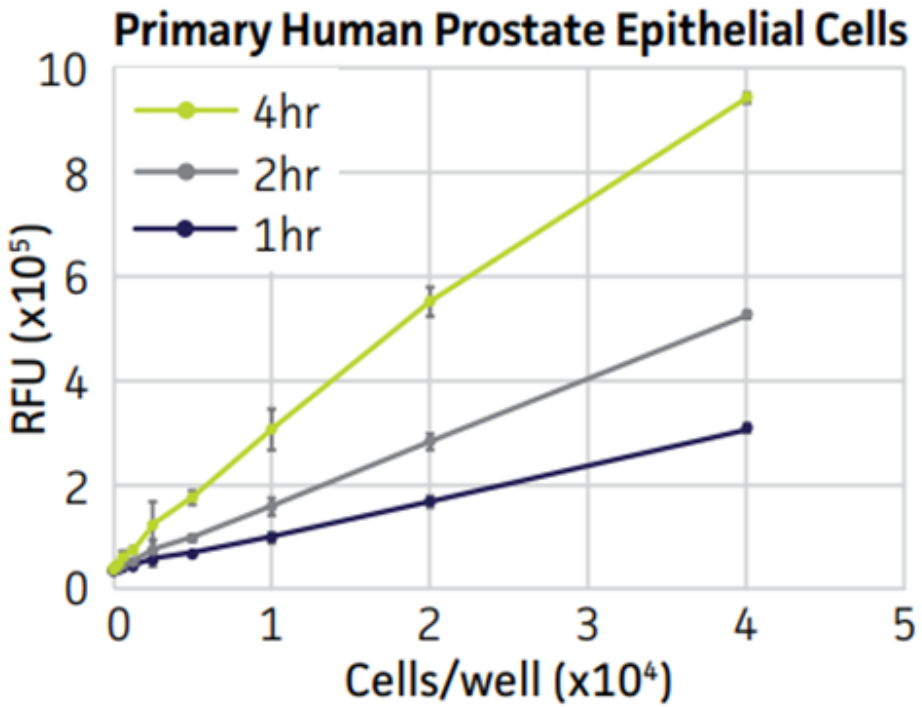


Reliablue™-derived viability curves

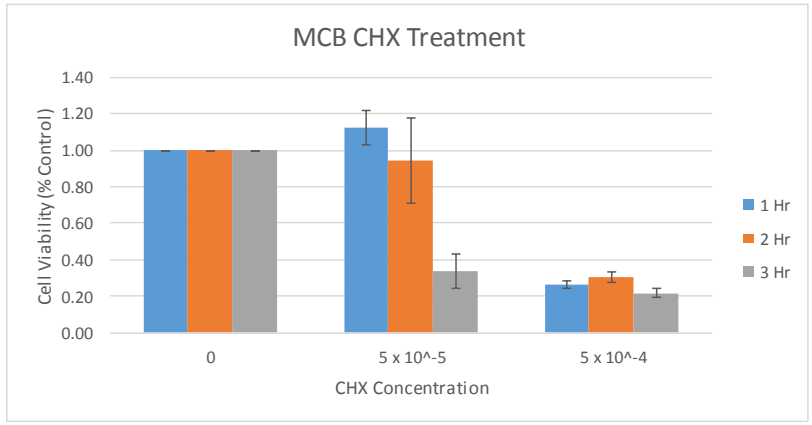


Reliablue™-derived viability curves

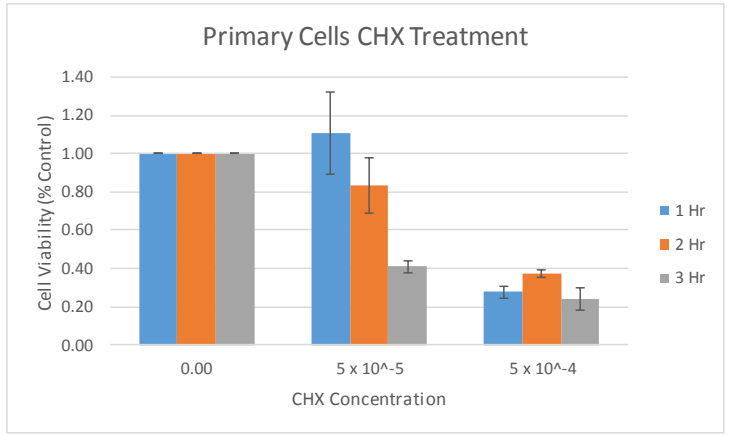




Reliablue™-derived viability curves



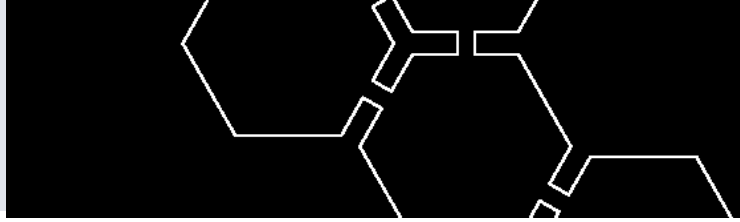
hTERT lung fibroblast (ATCC® CRL-4058™)

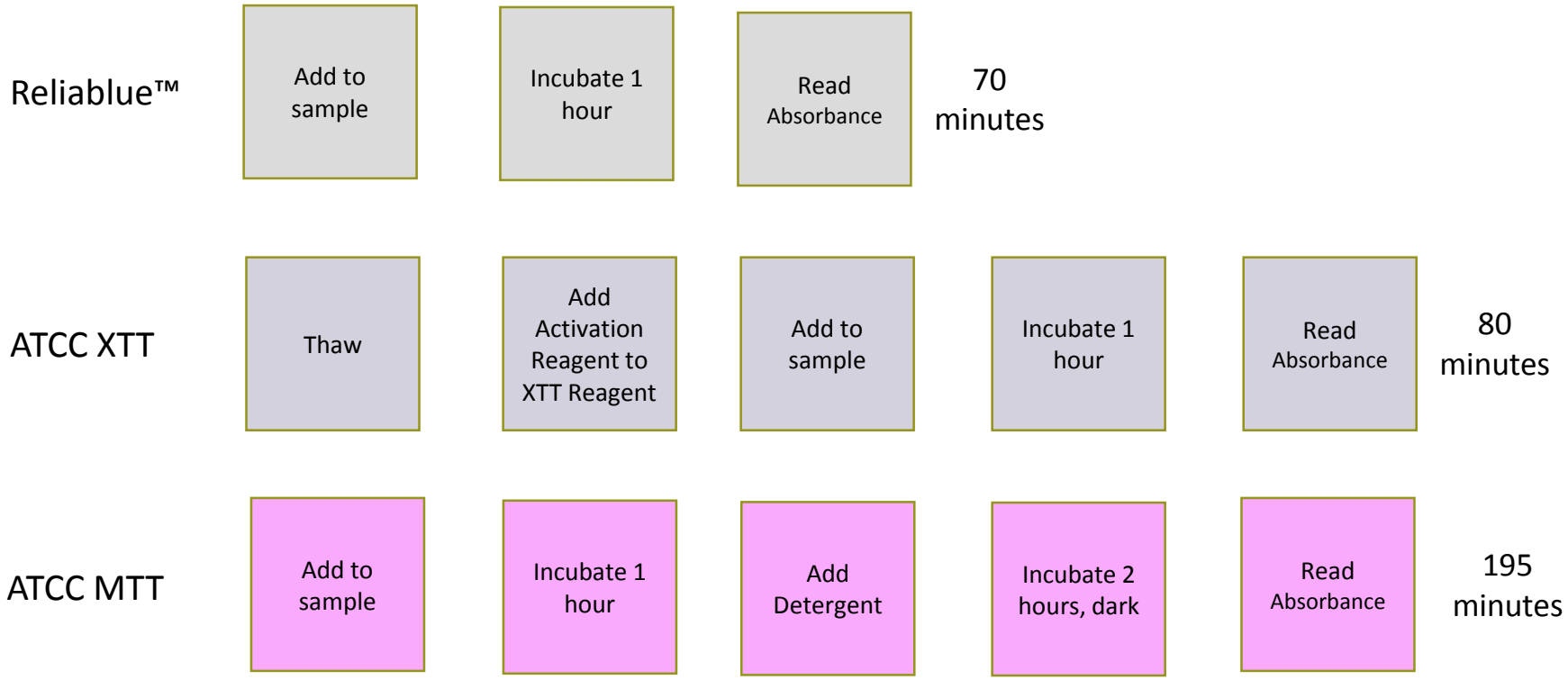


Primary Lung Fibroblast (ATCC® PCS-201-013™)

Primary & hTERT-immortalized lung fibroblast cells were seeded at identical numbers in a 96 well plate. The effect on cell viability of two concentrations of CHX (5.0×10^{-5} & 5.0×10^{-4}, microliters of CHX to microliters of cell culture media) was tested at three time points (1, 2 & 3 hours). Untreated cells were used as a negative control. One hour prior to the each time point Reliablue™ Reagent (ATCC® 30-1014™) was added to wells and allowed to develop, absorbance was then measured after an hour. Absorbance of treated cells was then compared to negative control cells to determine the percentage of live cells. All samples were done in triplicates.

Reliablue™ vs MTT and XTT





Reliablue™

- Quick, one step, one reagent
- Nontoxic
- Inexpensive
- ***HIGH-THROUGHPUT SCREENING***

Outline

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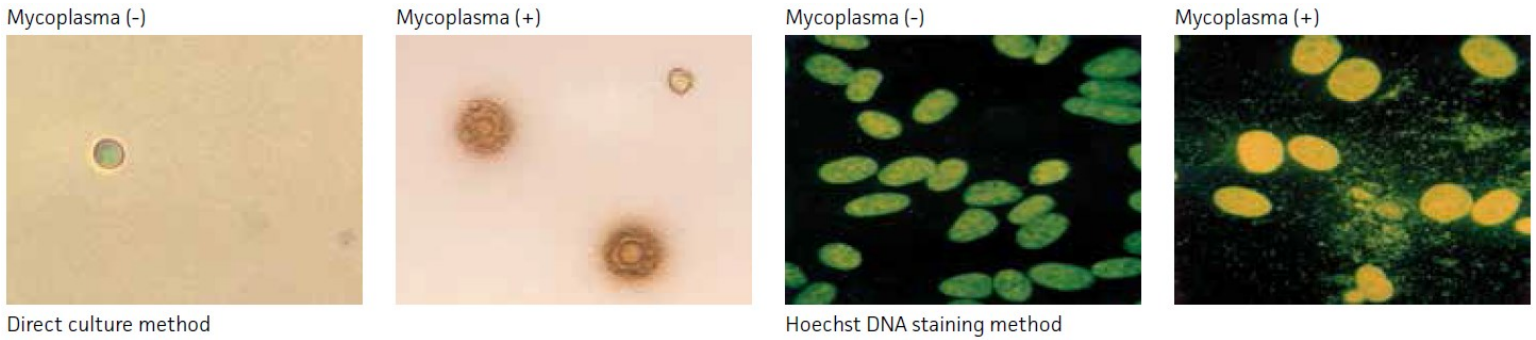
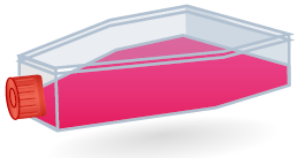
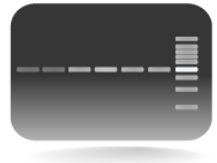
- **Mycoplasma Effects / Detection**

Mycoplasma contamination

- Not easily detected, cannot be seen by microscopy
- Chromosomal aberrations
- Disruption of nucleic acid synthesis
- Changes in membrane antigenicity
- Inhibition of cell proliferation and metabolism
- Decreased transfection rates
- Changes in gene expression profiles
- Cell death



Universal Mycoplasma Detection Kit ATCC® 30-1012K™

Grow cells	Scrape and suspend cells, or count	Add Lysis Buffer	Perform PCR	Run gel
				
Works with adherent and suspension cells. Use 10^4 to 10^5 cells per assay.	Transfer cell suspension to Sample Lysis Tubes. Pellet cells. Cell pellet can be stored until ready to assay.	Incubate. Heat at 95°. Spin. Transfer cell lysate to new microfuge tube. Cell lysate can be stored until ready to assay.	Perform optimized touchdown PCR using Universal PCR + Primers Mix.	Clear results in less than 3 hours.

- Detects over 60 species of *Mycoplasma, Acholeplasma, Spiroplasma,* and *Ureaplasma*
- All components for the PCR reaction are provided and optimized for amplification

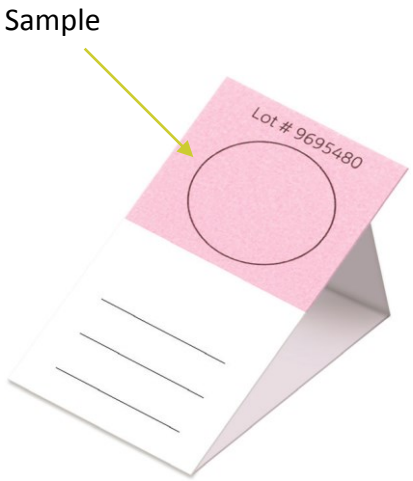
Mycoplasma testing service

Direct and indirect culture (bundled service)

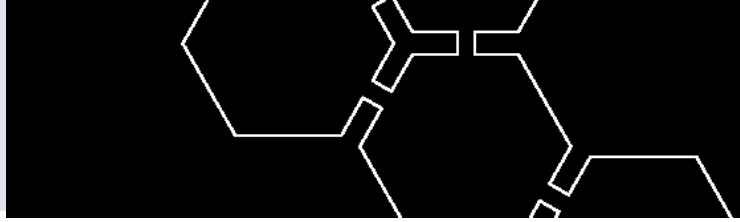
- Direct culture – Uses both broth and agar
- Indirect culture – Hoechst DNA stain

PCR-based testing

- Detection using the ATCC Universal Mycoplasma Detection Kit
- Newer, quicker method using FTA paper
- FTA lyses cells on contact, protects from degradation
- ISO/IEC 17025:2005 certified
- 3-5 business days

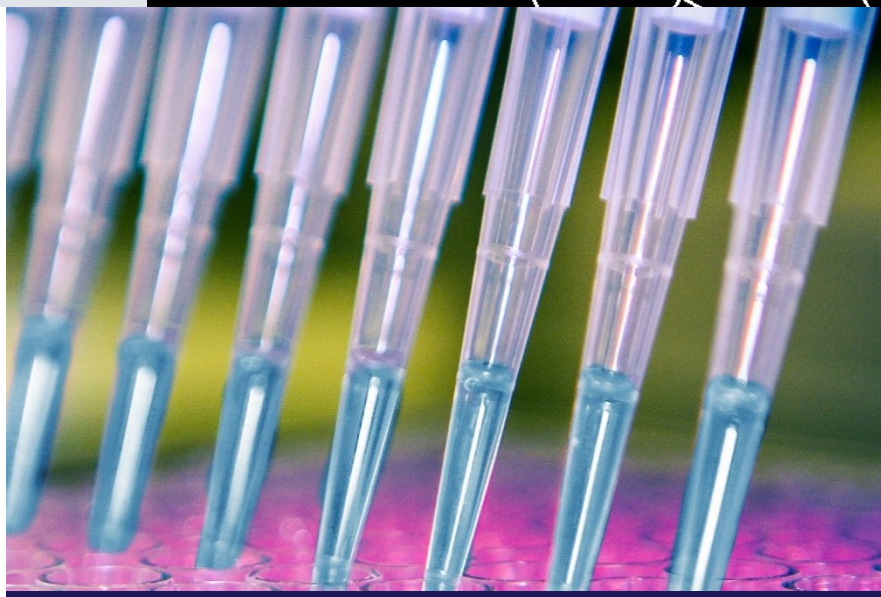


Summary points



Cell culture	• Select appropriate media for your cells • Understand issues/considerations for adding additional ingredients • Consistently use same media whenever possible
Cryopreservation	• Use a reliable rate-controlled cooler • Keep mammalian cells at -140°C for long term • Keep cells in the vapor phase in liquid nitrogen tanks
Measuring Cell Viability	• Importance of understanding growth rates in cell culture • Use of viability assays and reagents in measuring proliferation
Mycoplasma Detection	• Mycoplasma can cause cell death or inhibit proliferation and viability • Routinely check for mycoplasma in cell cultures

Back-To-School Savings



Buy two continuous cell lines, get one Reliablue™ Cell Viability Reagent free

- or -

Save 30% off your next purchase of Reliablue™ Cell Viability Reagent

Don’t wait too long to order! Our sales event ends on **October 12, 2018**.

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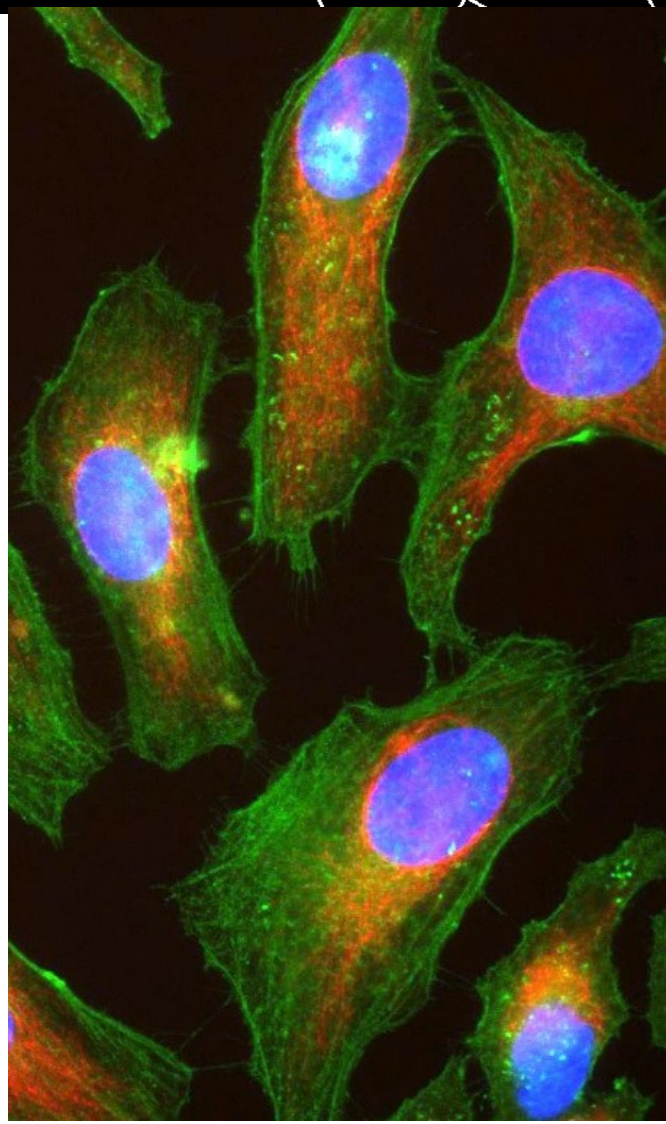

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