Cell Health and Viability

Steven Budd, M.S., M.B.A.
Product Line Business Specialist, ATCC

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees

Established partner to global researchers and scientists

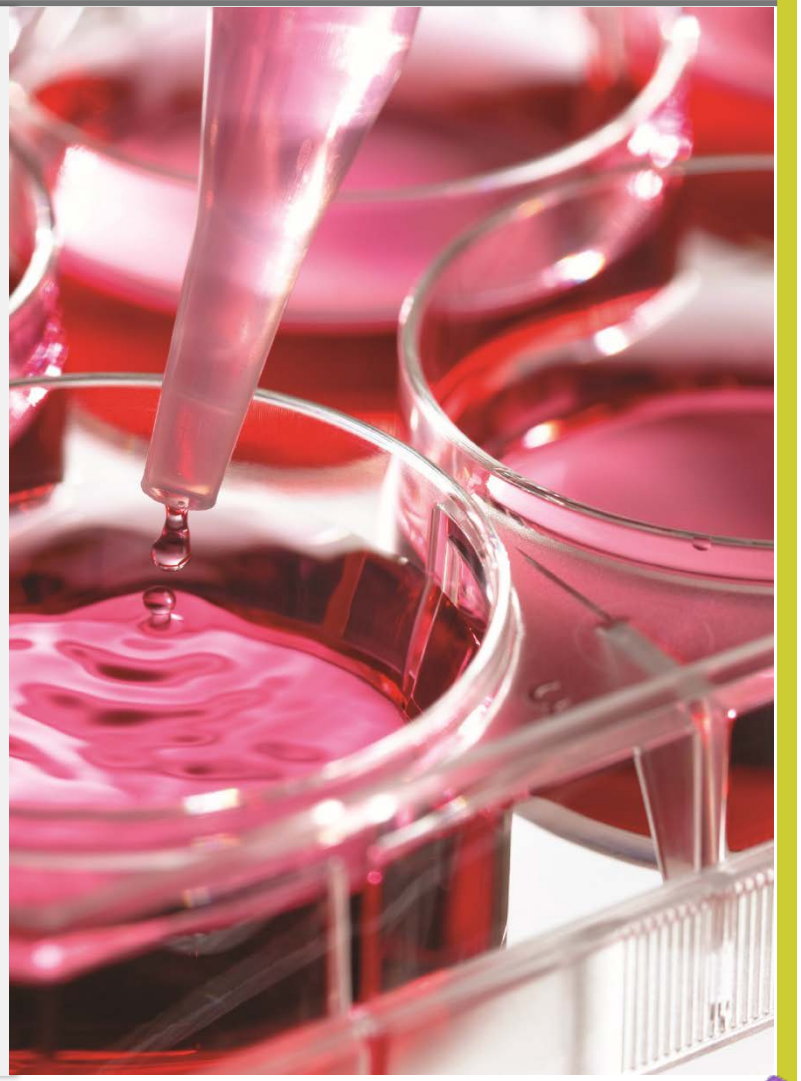
Outline

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

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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

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Media usage considerations

Maintain cells in the same media

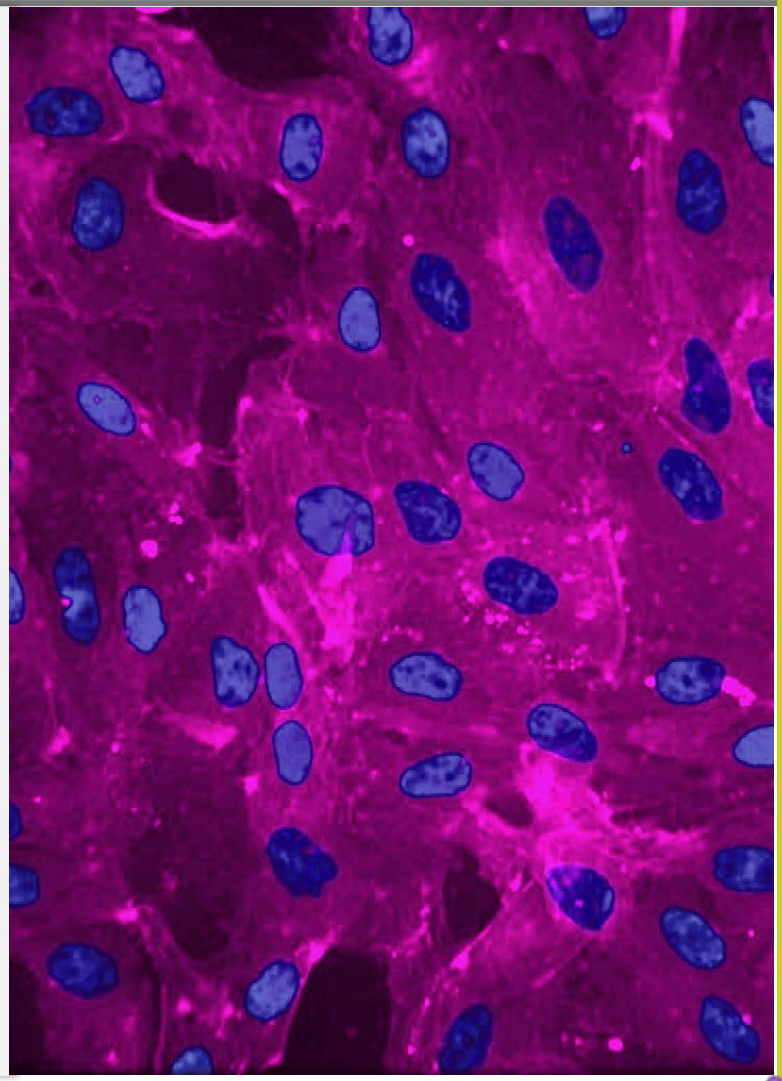
Media variability

- 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



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- **Cryopreservation / Post Thaw**
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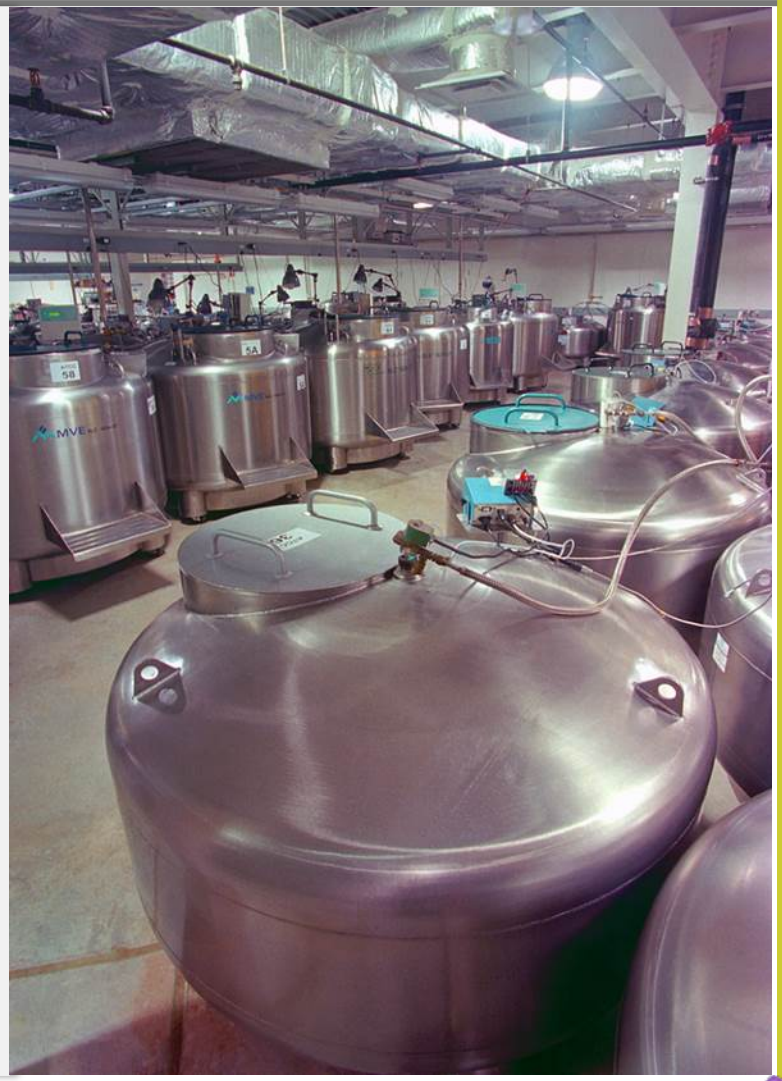
  - Reliablue™ cell viability reagent
- Mycoplasma Effects / Detection

Cryopreservation procedure

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



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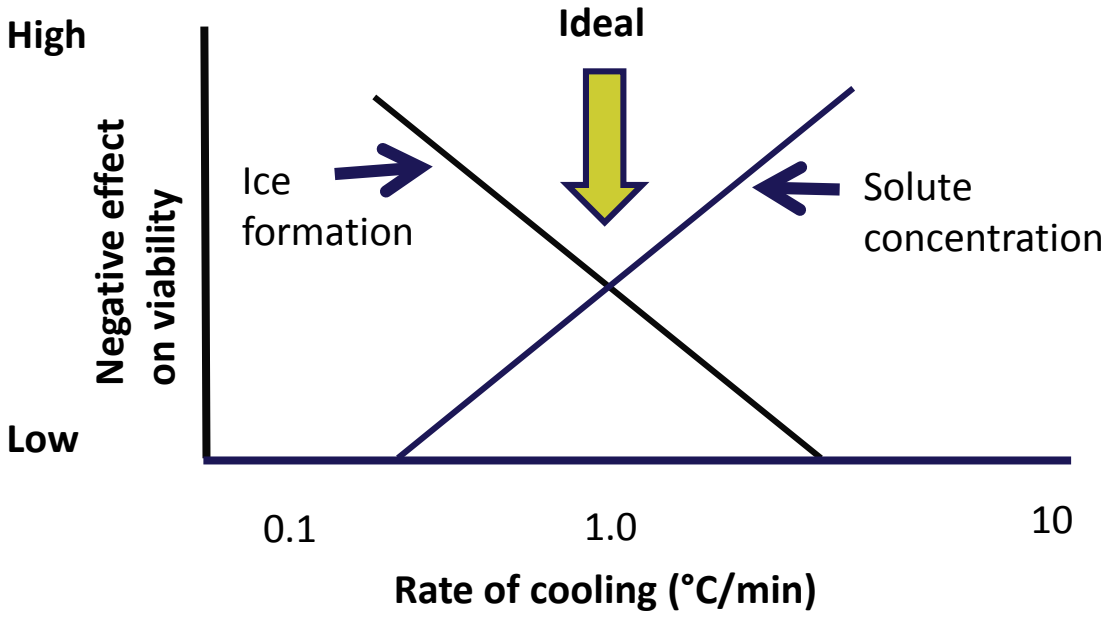
Cryopreservation principles



- 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	Number of cells
Animal cells	DMSO (5-10%) or Glycerol (5-10%)	-140°C	10^6 to 10^7/mL
Bacteria	Glycerol (5-10%)	-80°C	10^7/mL
Yeast	Glycerol (10%)	-140°C	10^7/mL
Protozoa	DMSO (5-10%) or Glycerol (10-20%)	-140°C	10^5 to 10^7/mL
Plant cells	DMSO (5-10%) and Glycerol (5-10%)	-140°C	3% to 20% cell volume
Animal viruses (free)	None	-80°C	NA
Animal viruses (infected cells)	DMSO (7%)	-10°C	10^6/mL

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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

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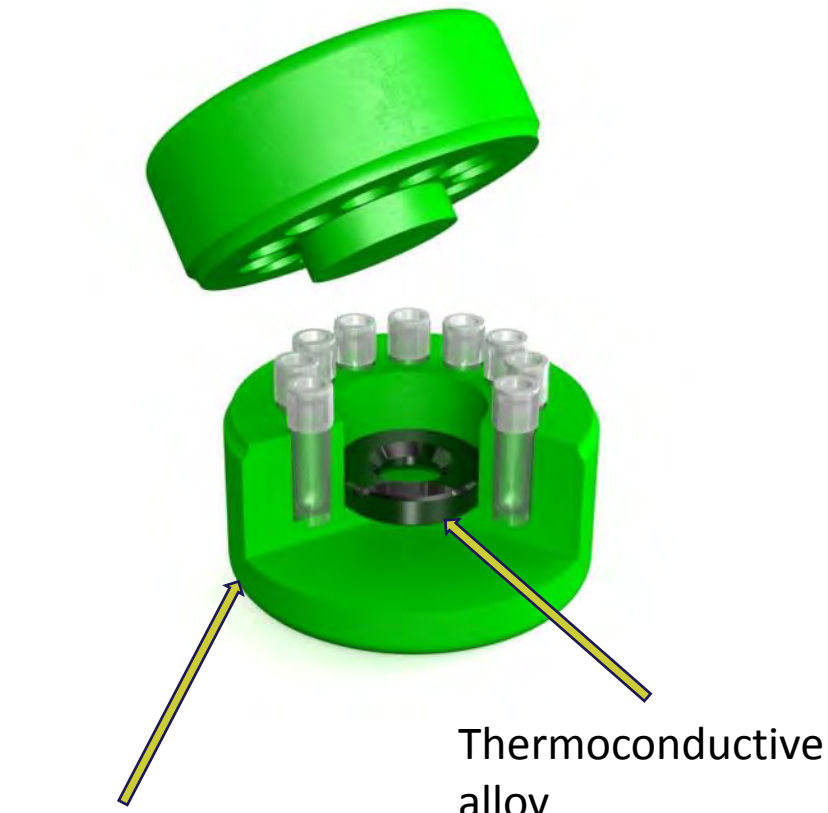
Freezing cells



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

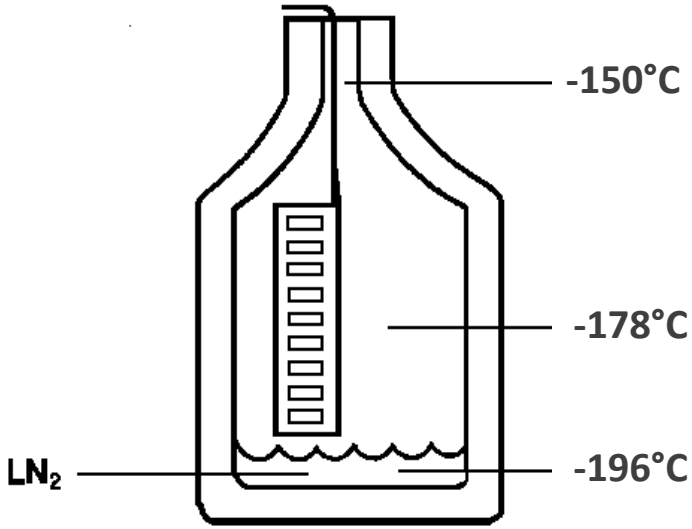
Mammalian cells

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 at the bottom of the tank

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



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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**

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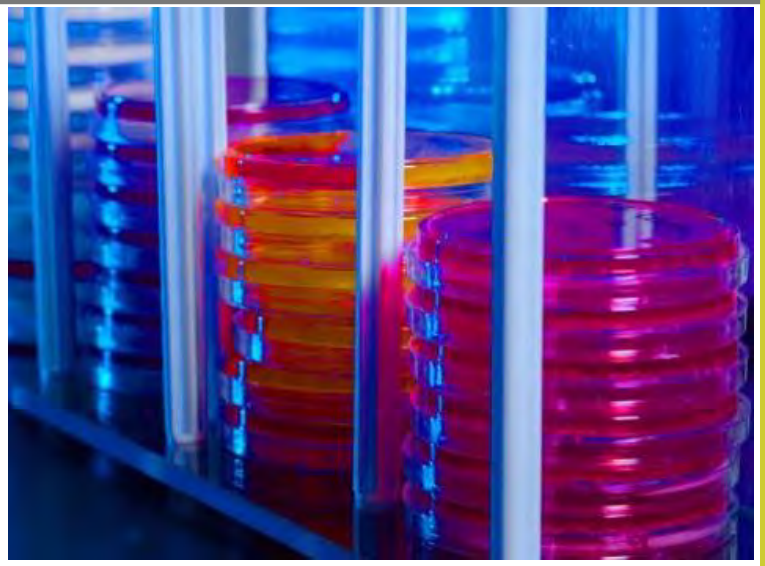
Cell expansion

- After thawing, cells should be plated in an appropriate cell culture vessel with complete media
- 24 hours after seeding, check for confluency

- **Note, primary cells may take up to several days to reach 80% confluency for subculturing**



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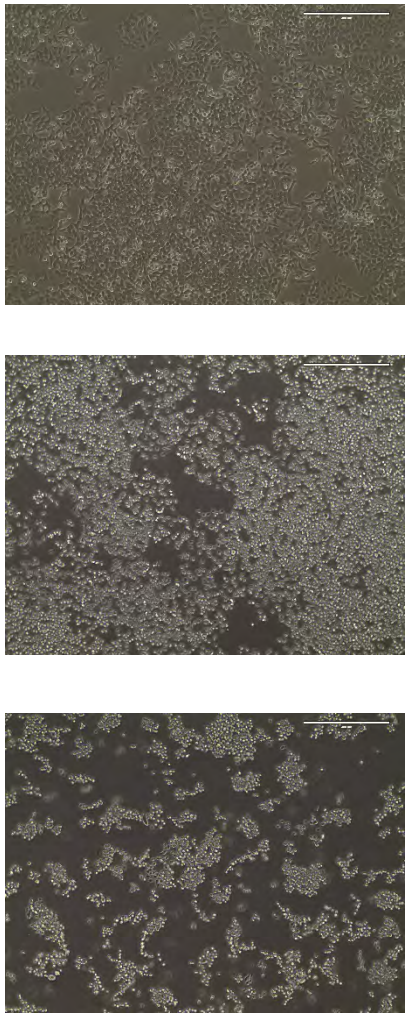
Trypsinization

At 80% confluency (primary cells), cells can be passed using Trypsin-EDTA

- Using warm trypsin-EDTA for about 3-5 minutes, cells will detach with gentle agitation
- ***Trypsin-EDTA for Primary Cells* (ATCC® PCS-999-003™) *is a low concentration formula (.05% Trypsin and .002% EDTA) – necessary for primary cell survival***
- A Trypsin Soybean Neutralizing Solution (ATCC® 30-2104™) is also needed to prevent cell damage

Monolayer



Fully trypsinized

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  - **Reliablue™ cell viability reagent**
- Mycoplasma Effects / Detection

Cell growth and propagation

- Population doubling level
- Measuring cell viability and growth
  - MTT Assay
  - XTT Assay
  - Reliablue™ Reagent

Population doubling level

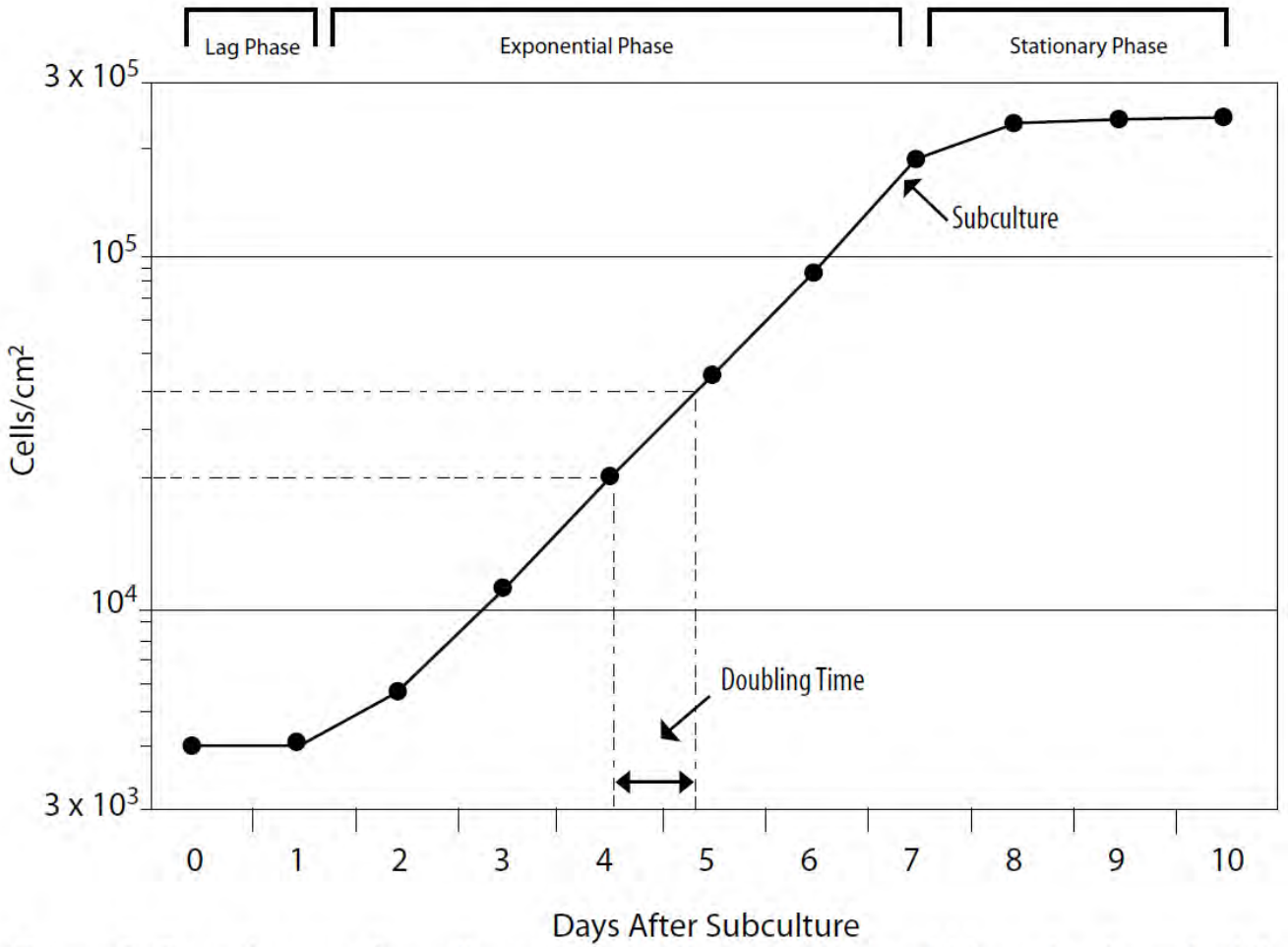

Figure 1. Growth curve for cells grown in culture. Cells should be subcultured while still in the exponential phase.

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Growth and viability

- Quantitative evaluation of **cell proliferation rate** and response to external factors that affect cell viability

- **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)**
  - Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide)

New!

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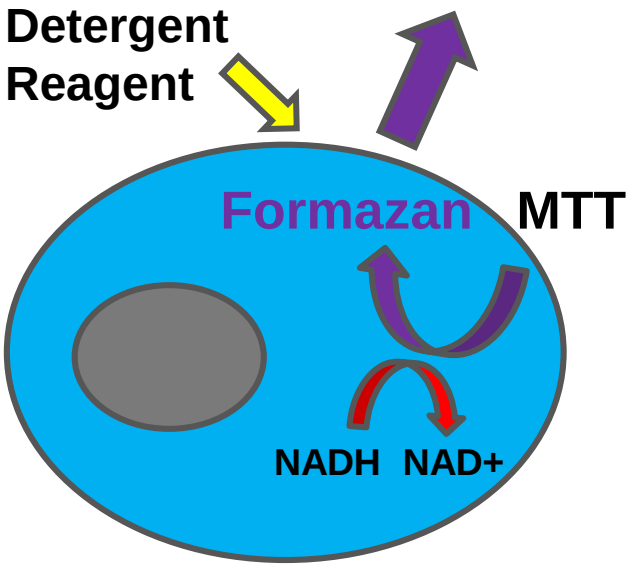
MTT / XTT

MTT Reaction

MTT salt is **reduced** within cellular matrix to Formazan, lysed with detergent to solubilize crystals

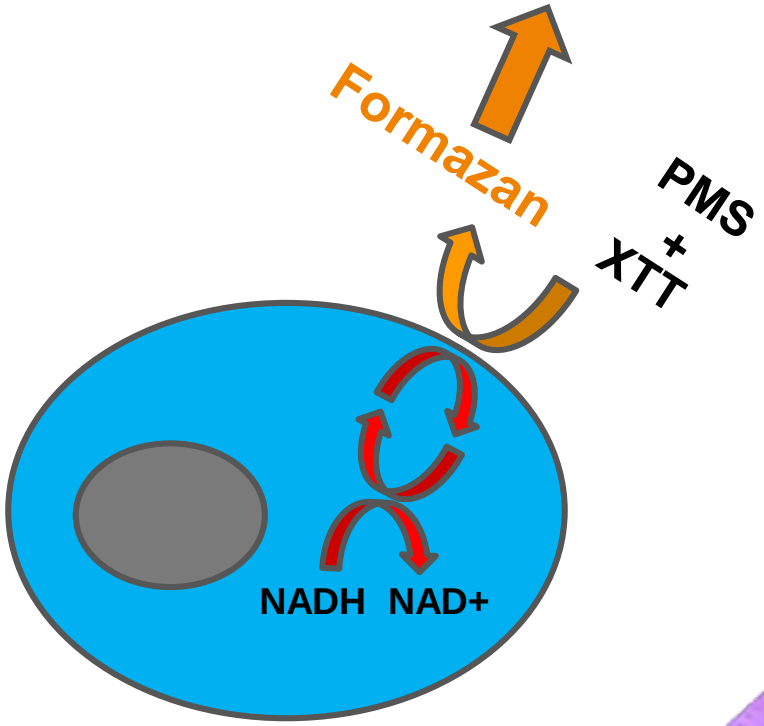
Media turns **PURPLE**



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

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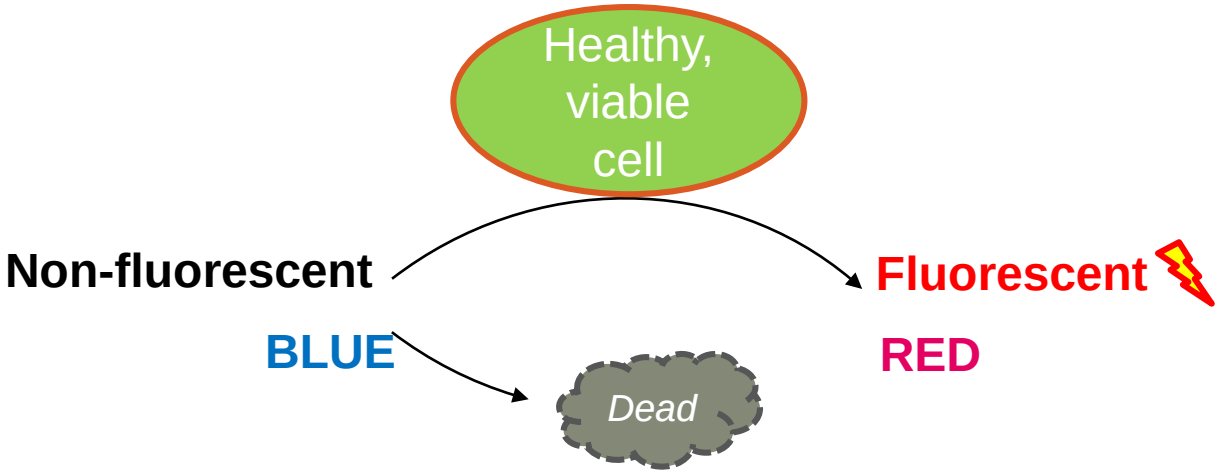
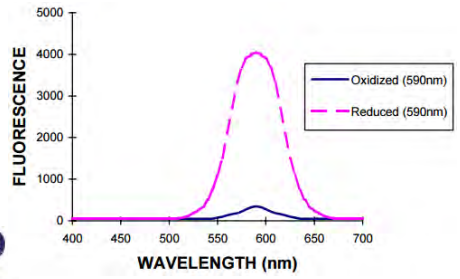
Reliablue™ Cell Viability Reagent

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.

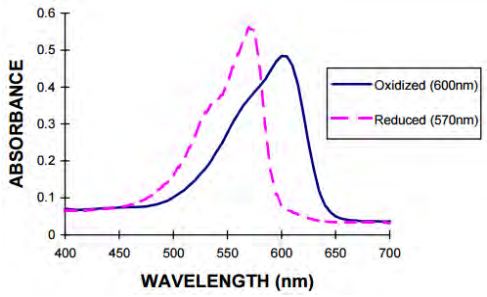


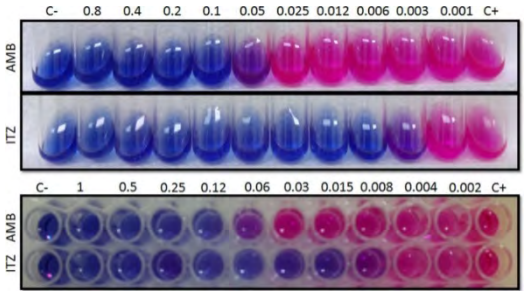
Resazurin is cell permeable but non-toxic and is metabolically reduced by living cells (but not dead cells or in the culture media) resulting in a change in absorbance and increase in fluorescence.

Fluorescence intensity



Absorbance Shift





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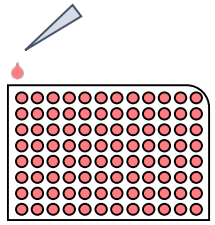
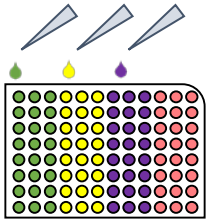
Reliablue™ Cell Viability Reagent

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.

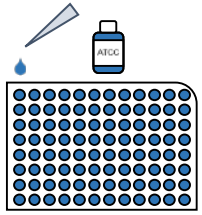
Basic 4-Step Assay Workflow



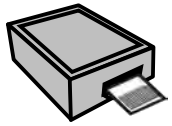
① Grow cells



② Add treatments



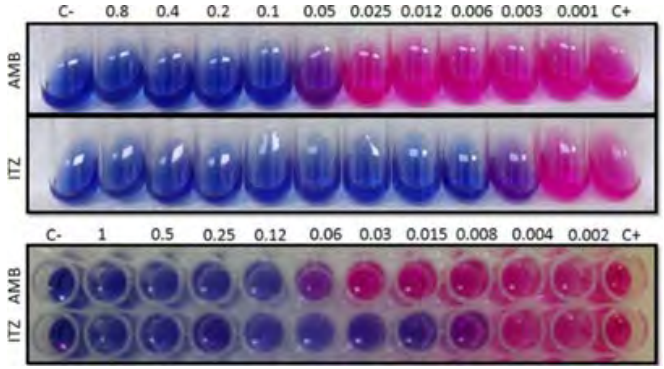
③ Add Reliablue



④ Read on plate-reader

Blue = Dead (or control)

Red = Alive and Actively Growing



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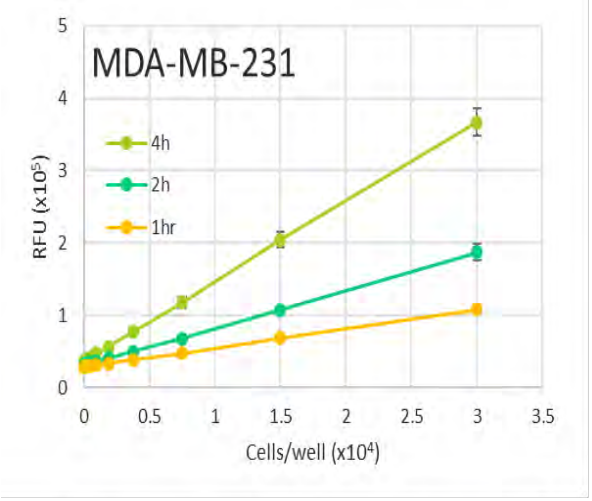
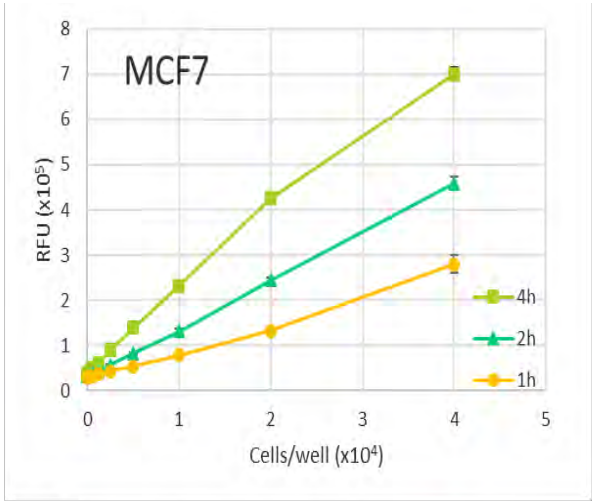
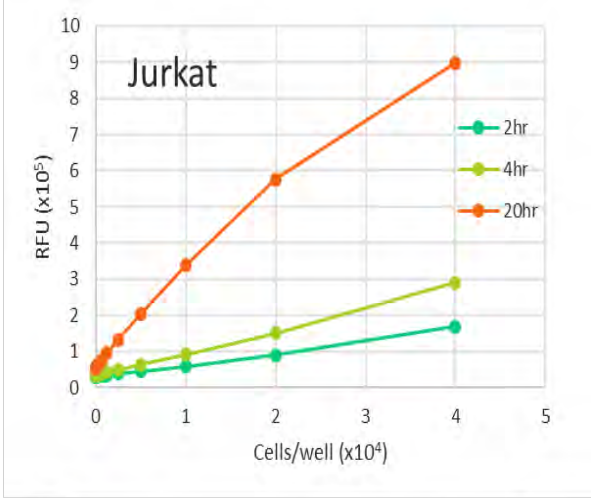
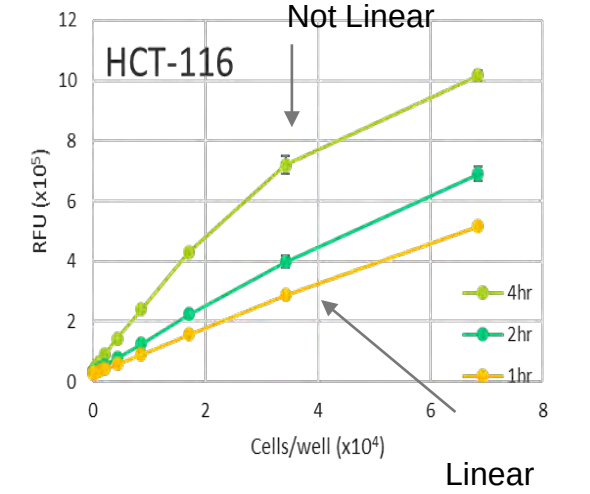
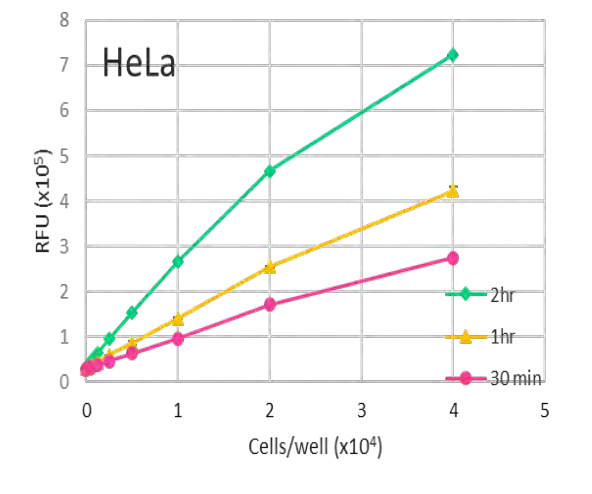
Cell Tested Reliablue™

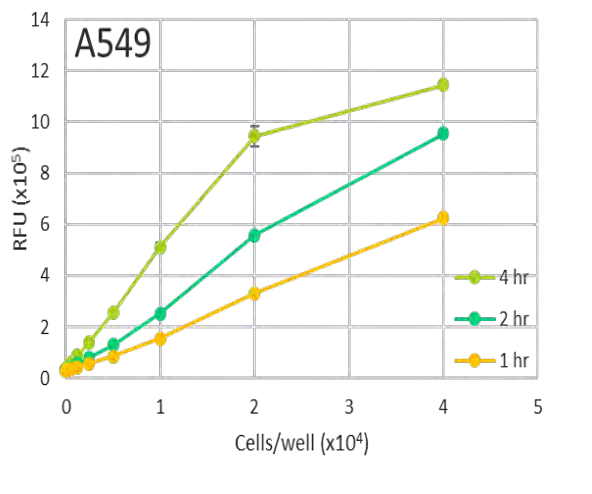












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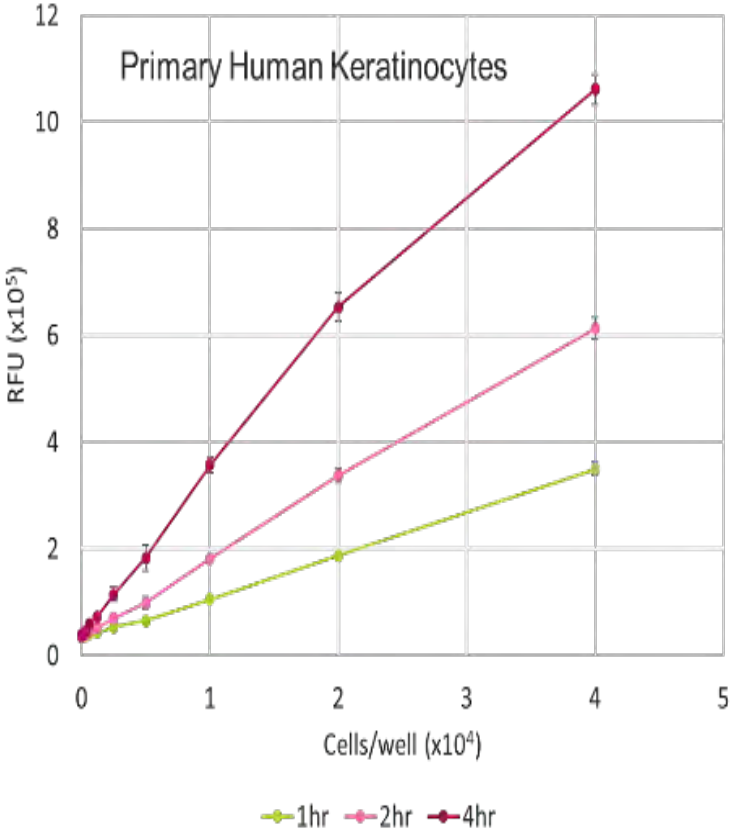
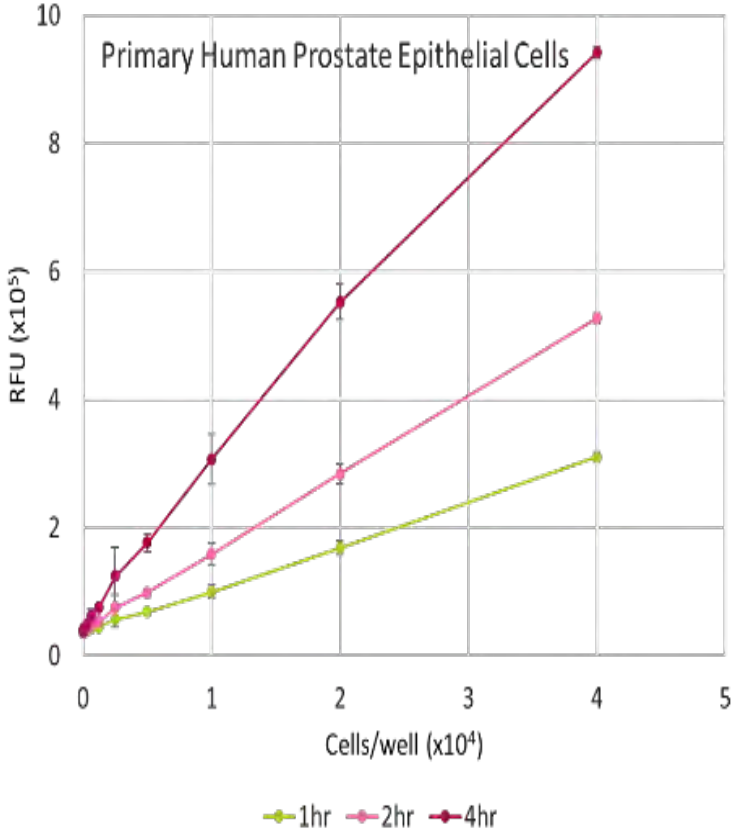
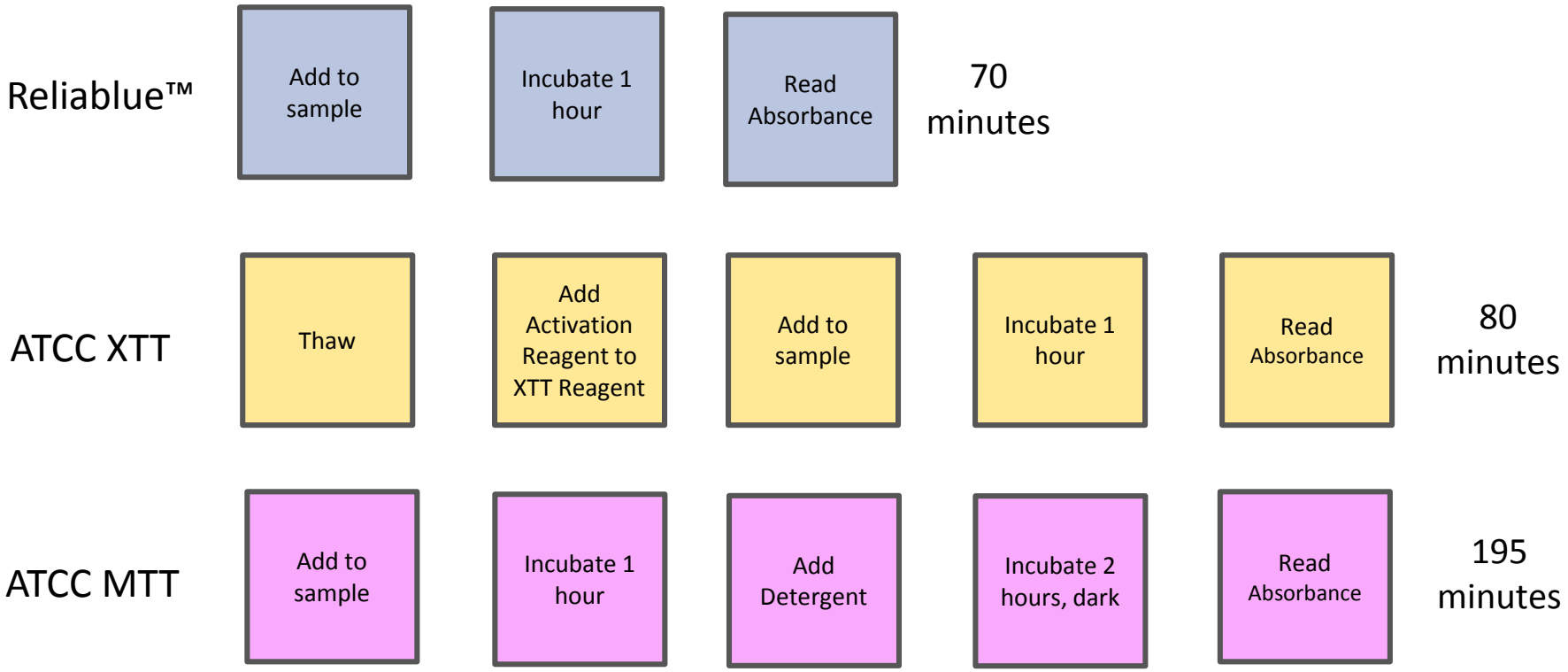




Reliablue vs. MTT / XTT Kit



Reliablue™

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

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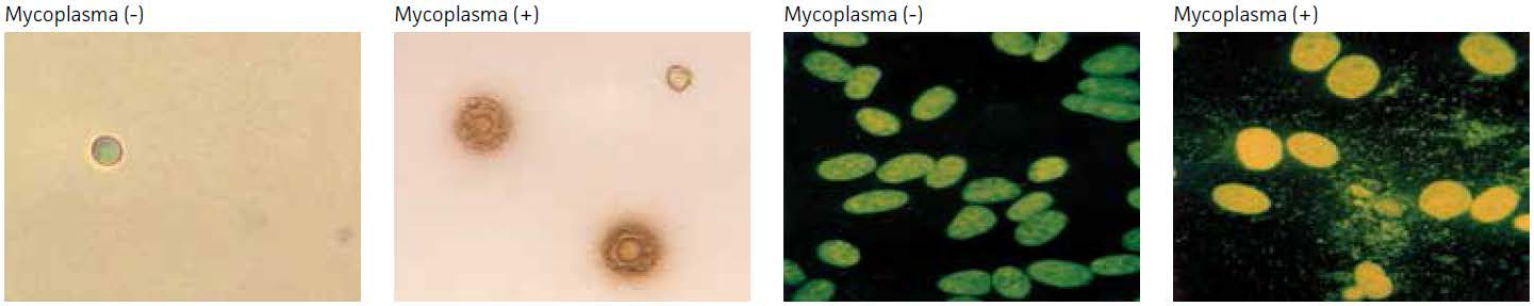
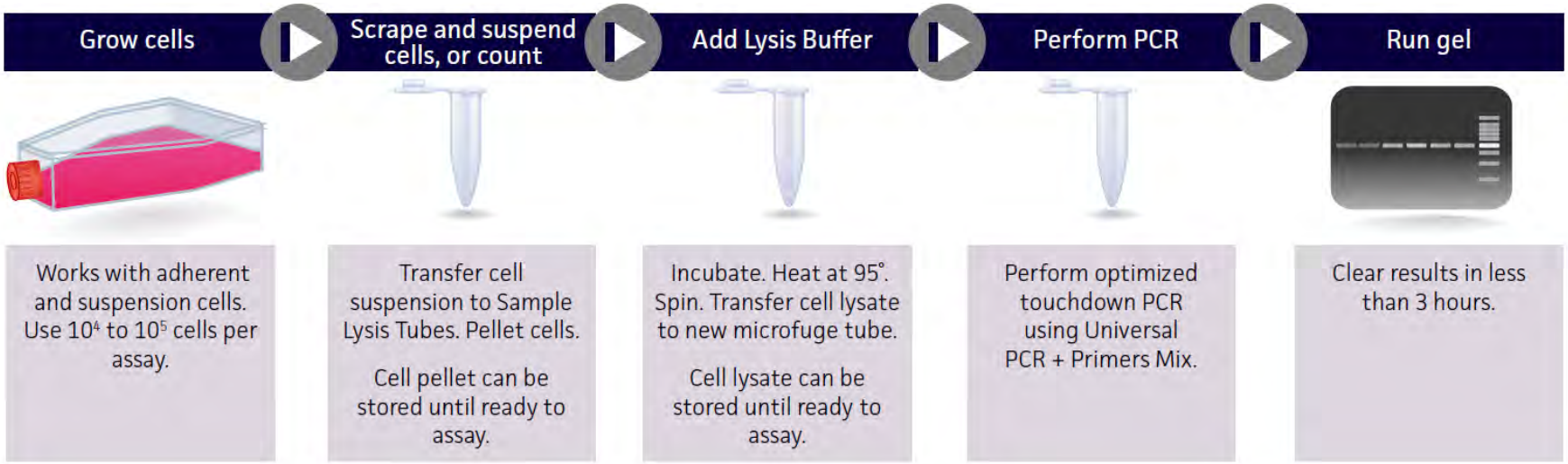
- **Mycoplasma Effects / Detection**

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™



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

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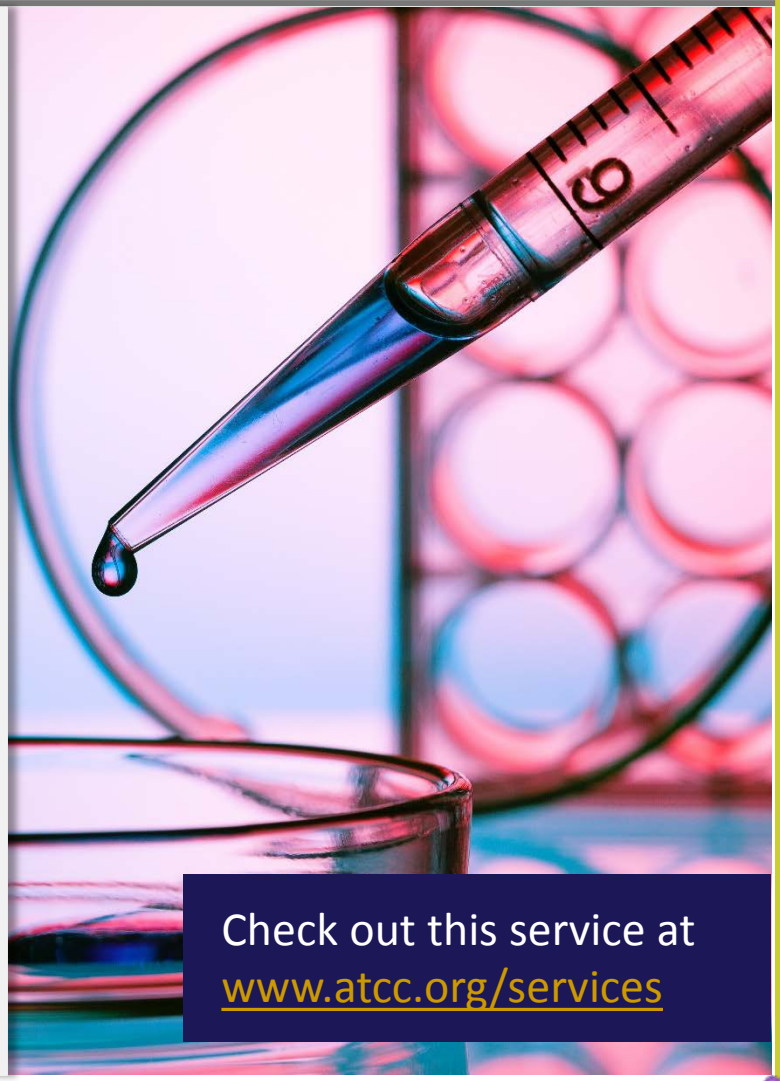
Mycoplasma testing service

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



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

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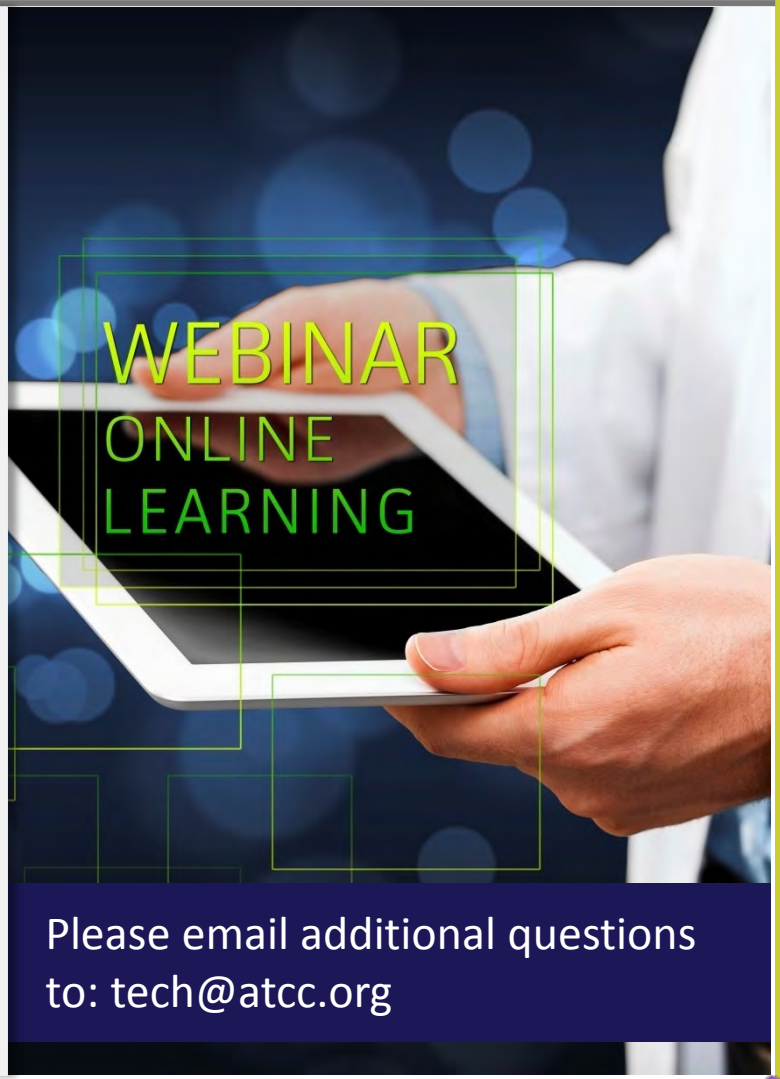

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