

Live in 3D (Cultures): Principles, News & Solutions

East Port & Promega Seminar

Lukáš Išler

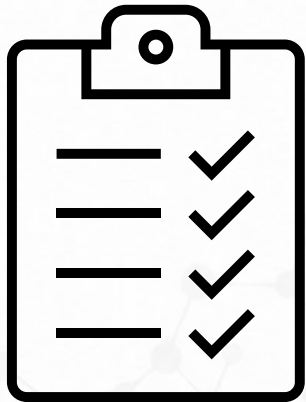
Application Specialist

October 2026

eastport.cz



Agenda



East Port overview & Introduction to 3D cultures (*Lukas Isler*)

Getting Information from Your 3D Cultures (*Kerem Yildirim*)

Coffee break, Q&A

Latest Technologies for Imaging 3D Models (*Vojtech Andrlle*)

News from East Port & Final thoughts



About East Port

- Over **25 years** supporting life science research in the Czech Republic
- **Partnerships** with more than **15** leading biotechnology suppliers
- Team with scientific background



We Provide



Biological Reagents and Instrumentation



**Scientific Application Support
Seminars & Webinars**



Technical Service



„Mini“ Seminars for Scientific Groups

- Personalized **seminars tailored to your lab needs**
- Part of our customer service → **free!**
- A few **examples** from the past:
 - Seminar for nanotoxicology group
 - Seminar with solution for CAR-T



Covered Product Areas



Molecular & Cell Biology (Oligonucleotides, Cell Health Assays, (q)PCR, Cultivation Media, DNA/RNA isolation, Enzymes etc.)



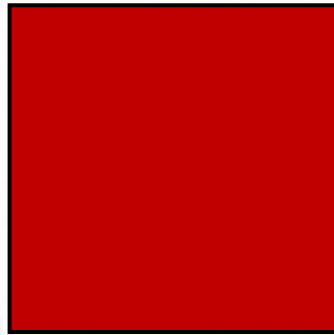
Microplate **Readers**, Cellular **Imaging** Systems, Automated **Nucleic Acid Extraction** etc.



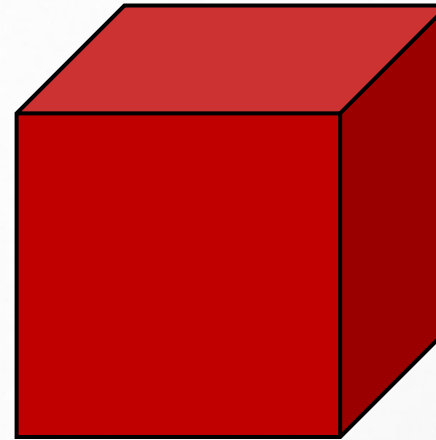
Pippette **tips**, (Micro)Centrifuge **Tubes**, **Microplates** and PCR Plates, **racks** etc.



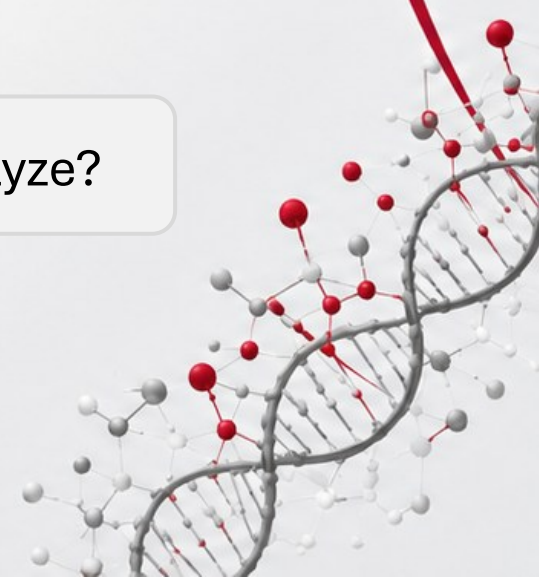
2D



3D

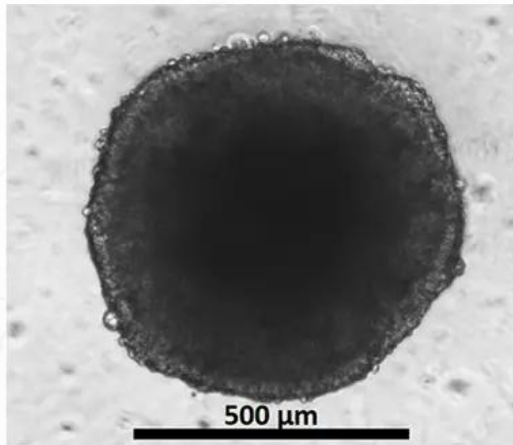


Why 3D? → What changes? → How to start? → How to analyze?

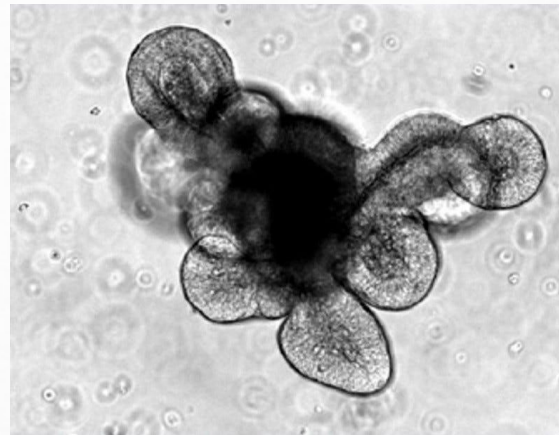


What are 3D cultures?

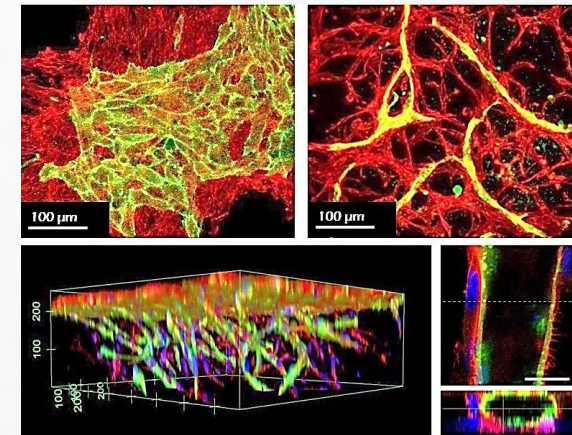
„3D cell cultures are in vitro models in which cells grow and interact in three dimensions“



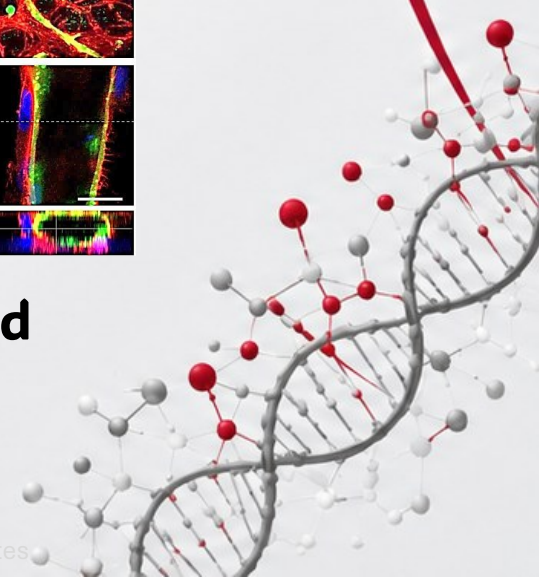
Spheroids



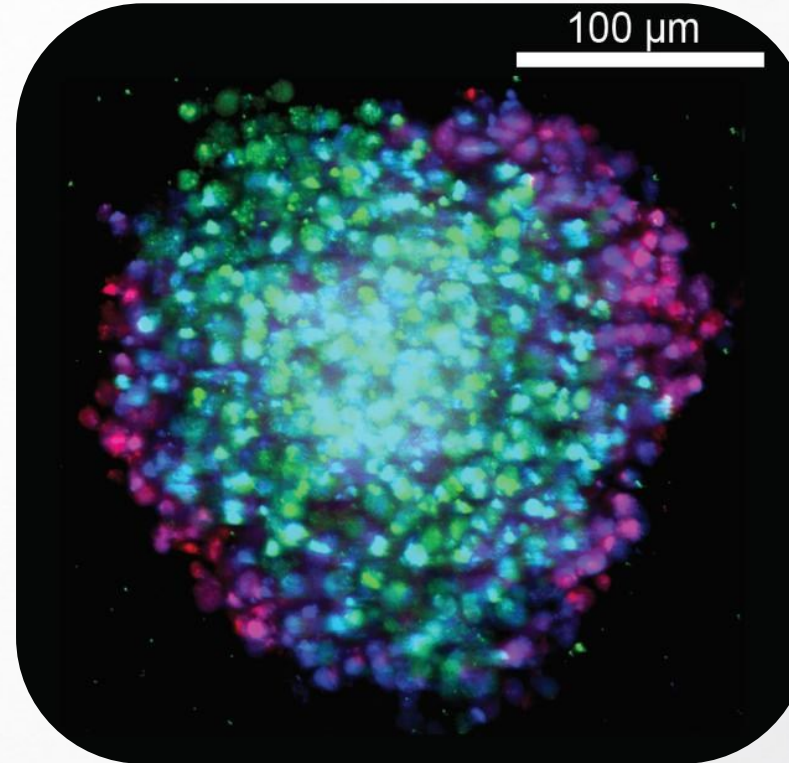
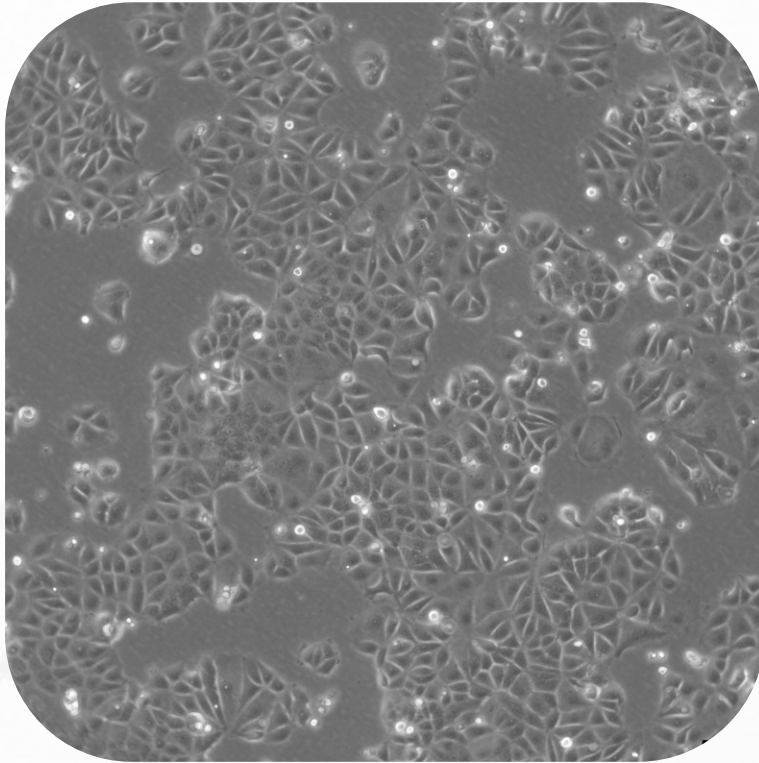
Organoids



Scaffold-based



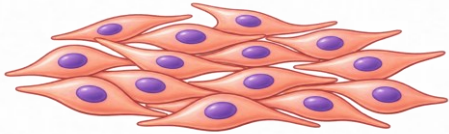
Why Do We Need 3D Models?



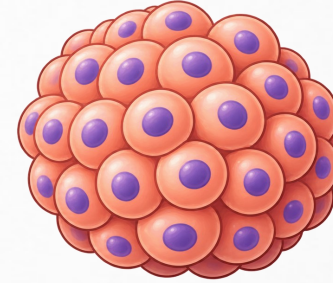
Do they respond differently?



Why Do We Need 3D Models?

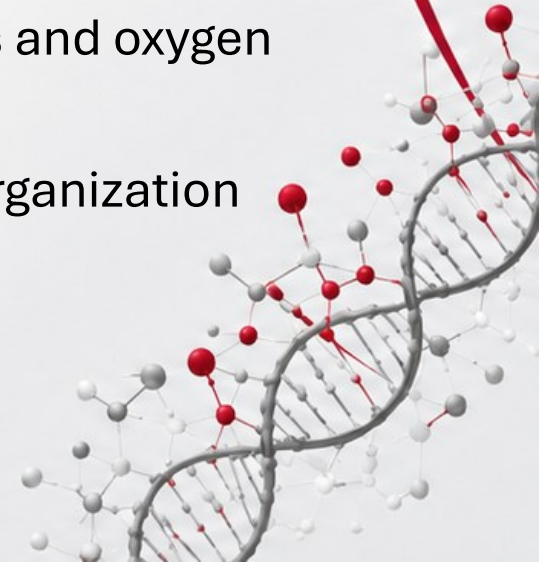


VS



- Non-physiological growth
- Uniform nutrients and oxygen
- Limited cell-to-cell and ECM interactions
- Drug response might be misleading

- Mimics tissue or tumor more closely
- Cellular heterogeneity
- Gradients of nutrients, metabolites and oxygen
- Natural cell-to-cell contacts and organization
- Natural interactions with ECM



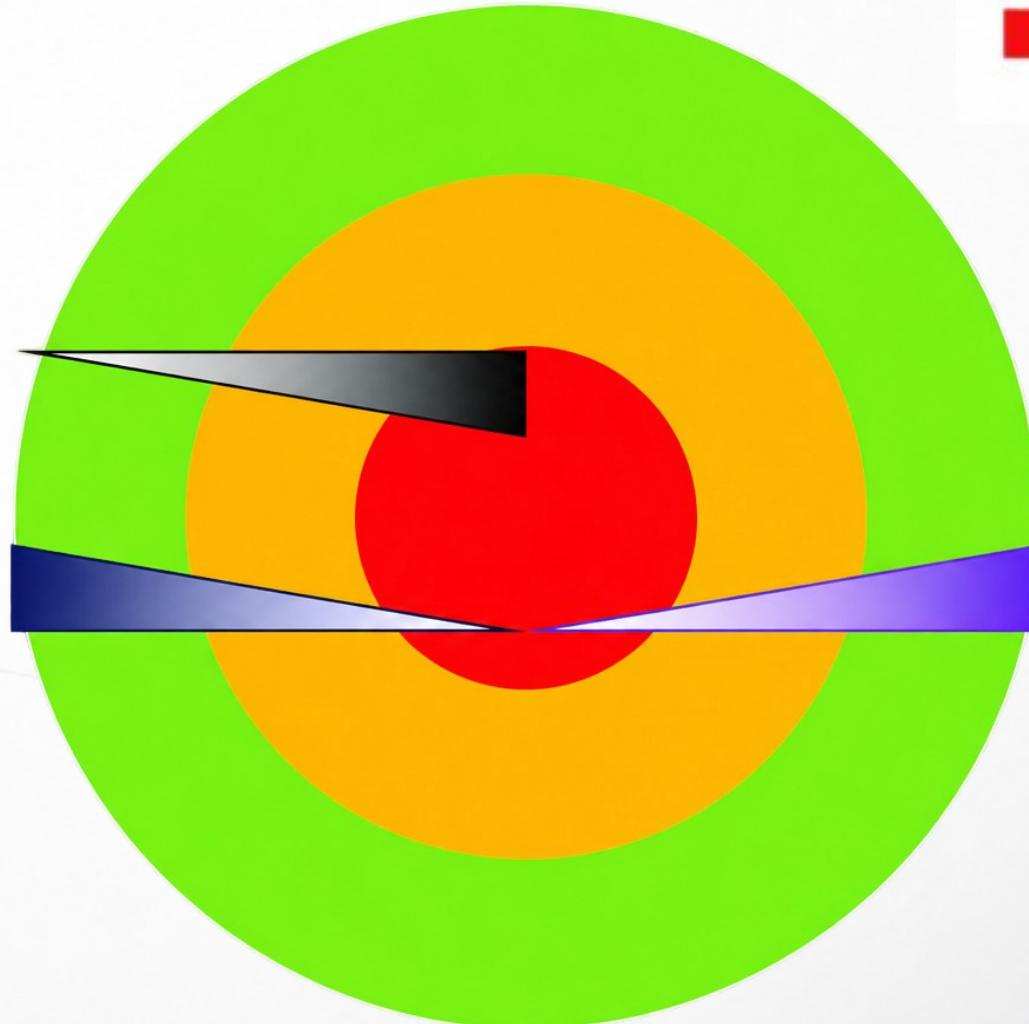
A Third Dimension Changes Biology

- Proliferating zone
- Quiescent viable zone
- Necrotic core

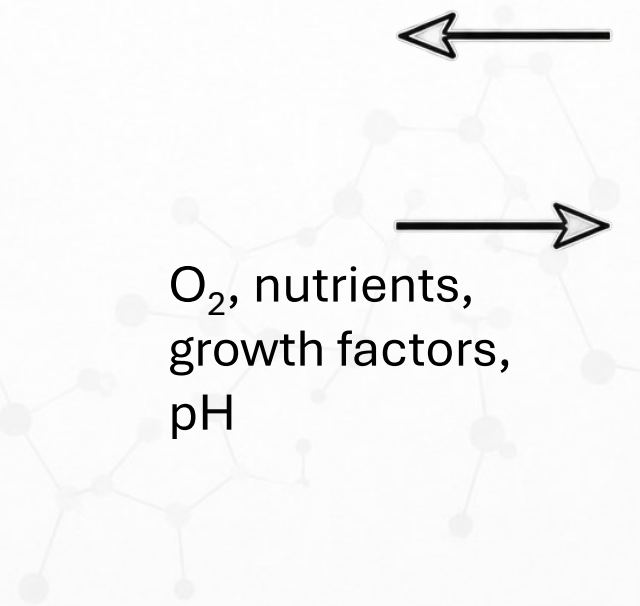
CO & Waste
products



O₂, nutrients,
growth factors,
pH



Drug / Reagent
penetration



Where Can 3D Models Make a Difference?

Cancer and drug discovery

Drug efficacy & penetration,
resistance, migration

Immuno-oncology

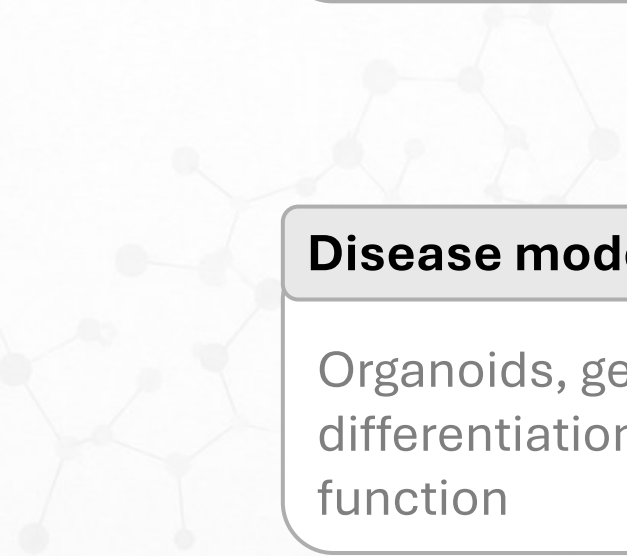
Immune cell infiltration, T-cell
and NK-cell killing

Disease modeling

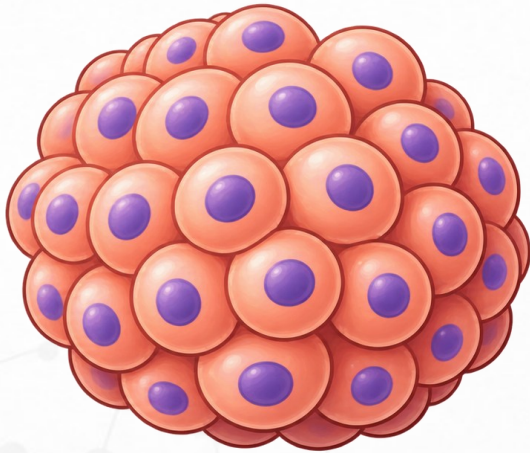
Organoids, genetic diseases,
differentiation and tissue
function

Toxicology

Tissue-specific responses,
chronic exposure and toxicity



Getting started with 3D cultures



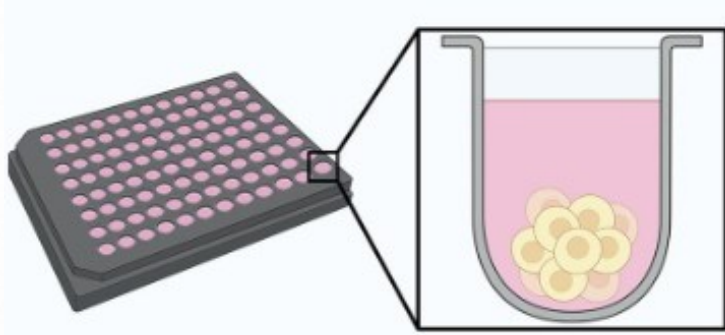
How to grow 3D?

First spheroid

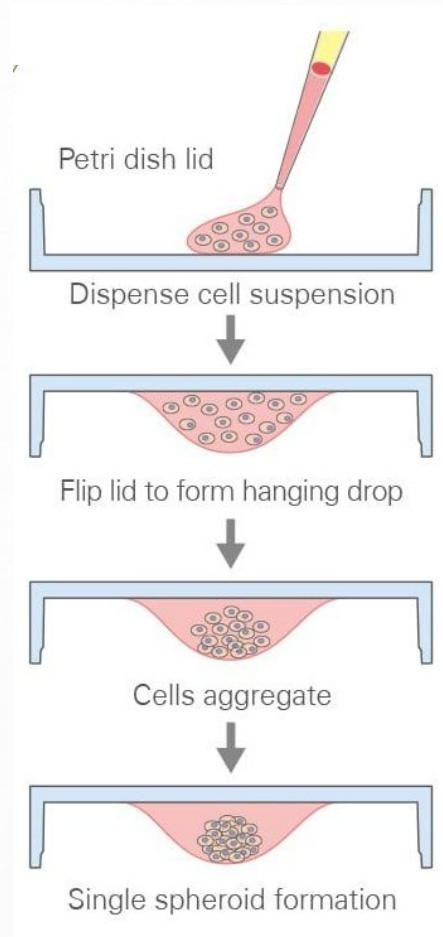
What can go wrong?



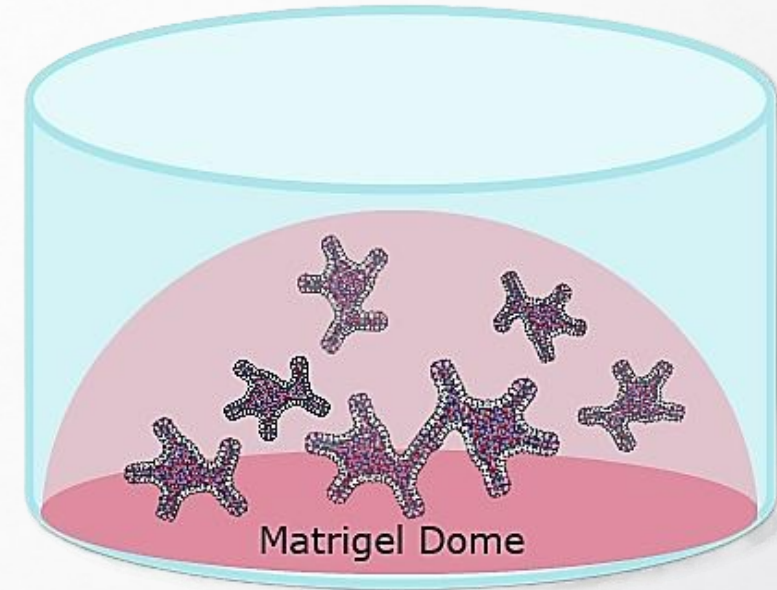
How Can We Grow Cells in 3D?



1) ULA Plates



2) Hanging Drops



3) Hydrogel-based

Creating The First Spheroid



CORNING



Corning® Spheroid Microplates
Spheroid Formation Protocol

Creating The First Spheroid

1) Prepare healthy cells

Start with a viable, well-dispersed single cell suspension

2) Seed cells into 3D plate

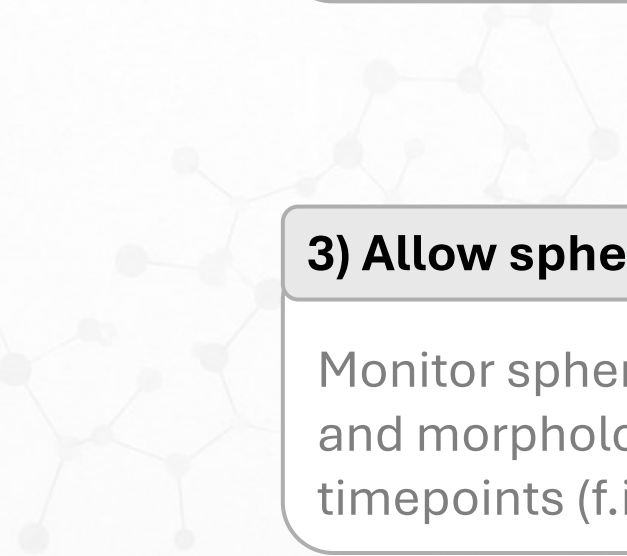
Choose different cell densities for optimization of spheroid formation

3) Allow spheroid to grow

Monitor spheroid formation and morphology in different timepoints (f.i. 24, 48 and 72h)

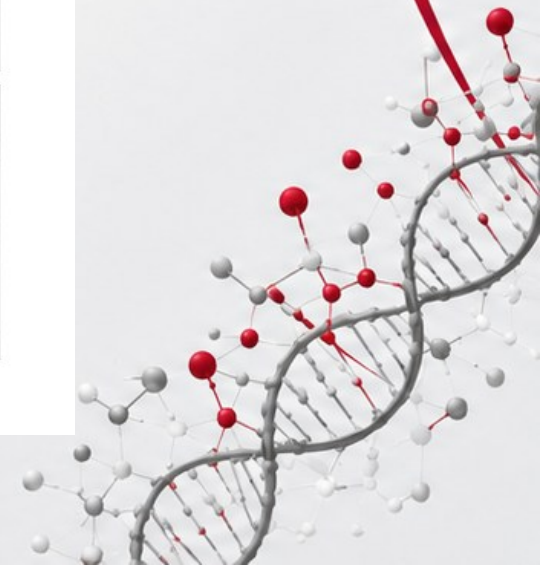
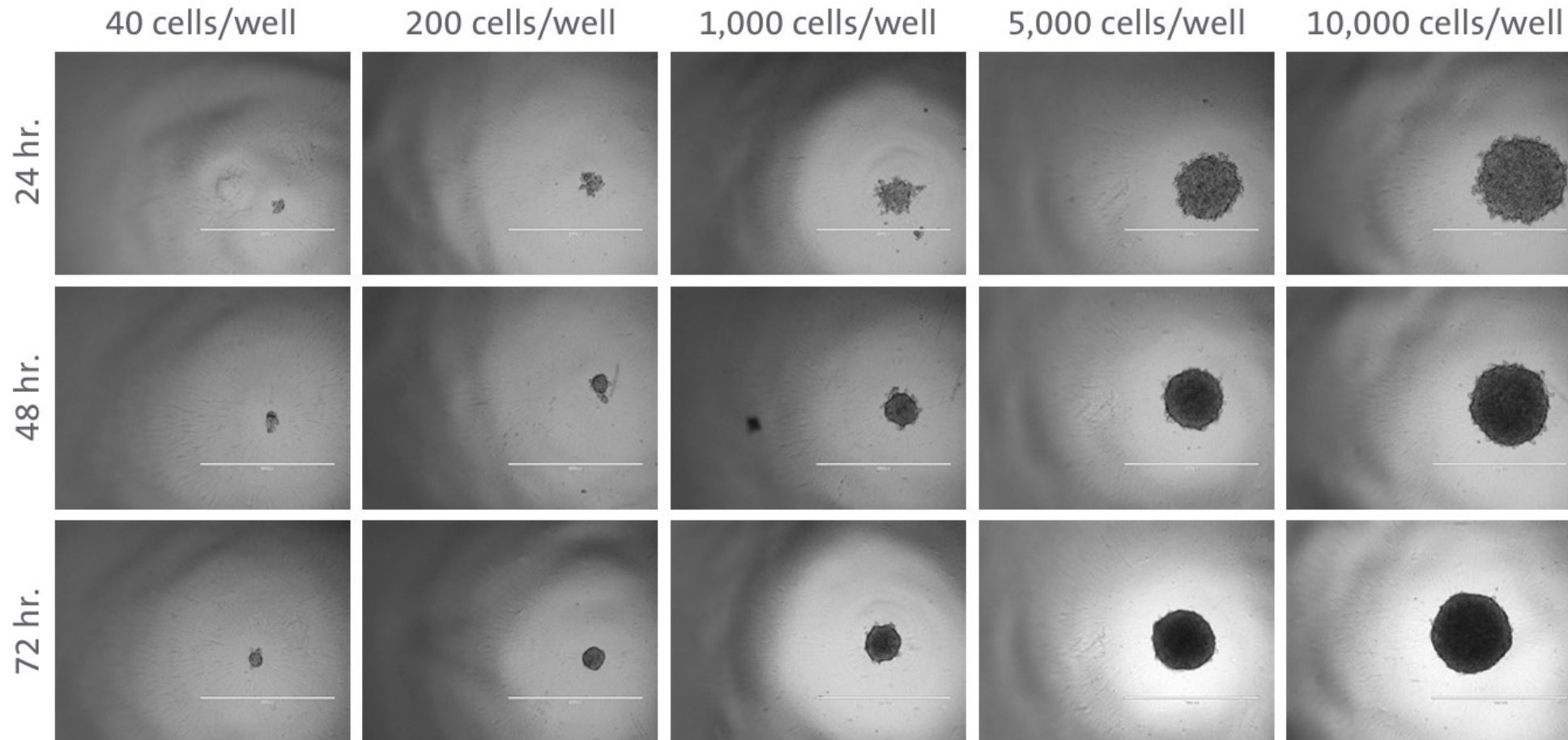
4) Check before treatment

Check size, morphology or viability

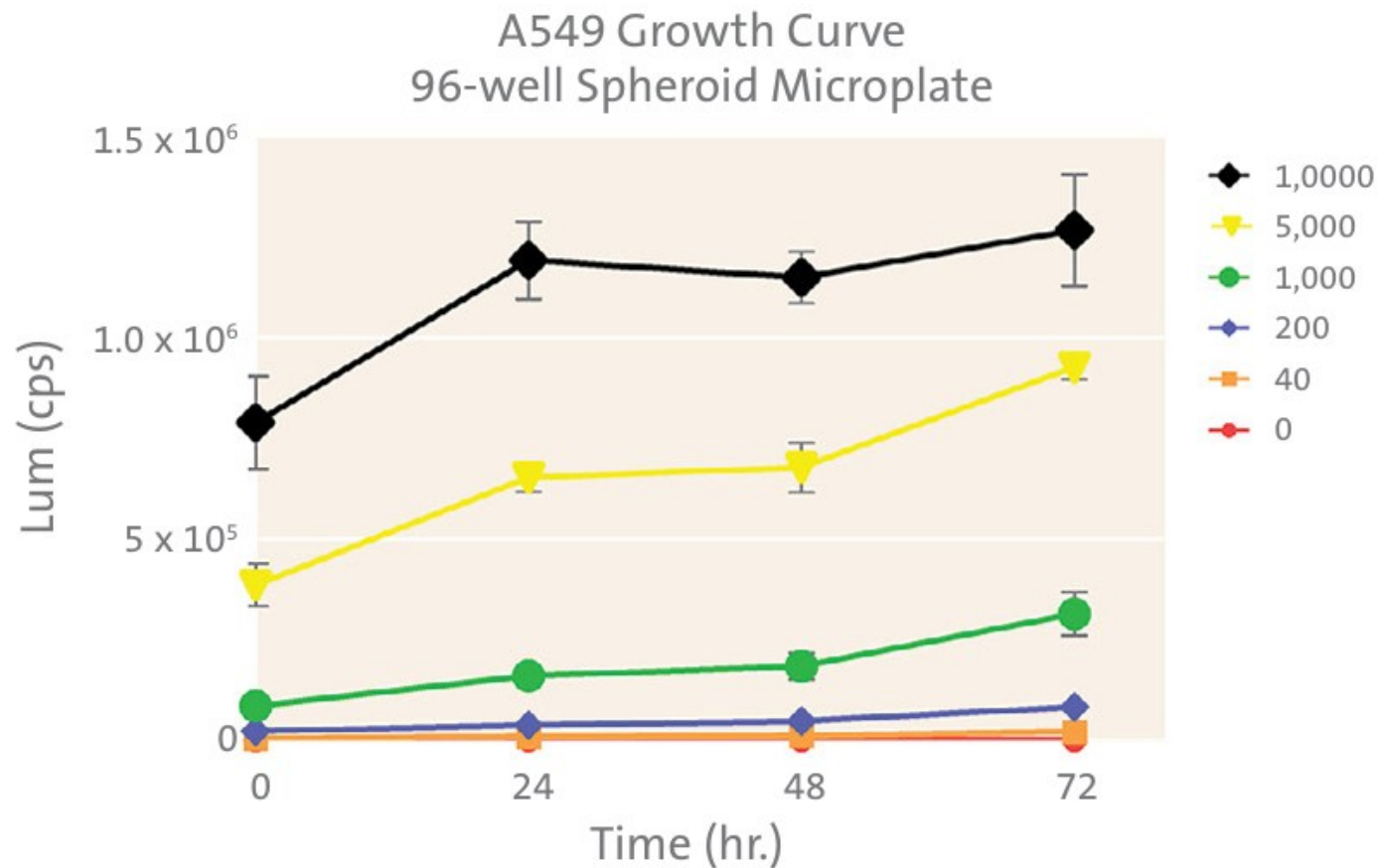


Creating The First Spheroid

A549 Spheroids



Creating The First Spheroid



CellTiter-Glo 3D Viability Assay

Spheroid Size Consideration



Small



Medium



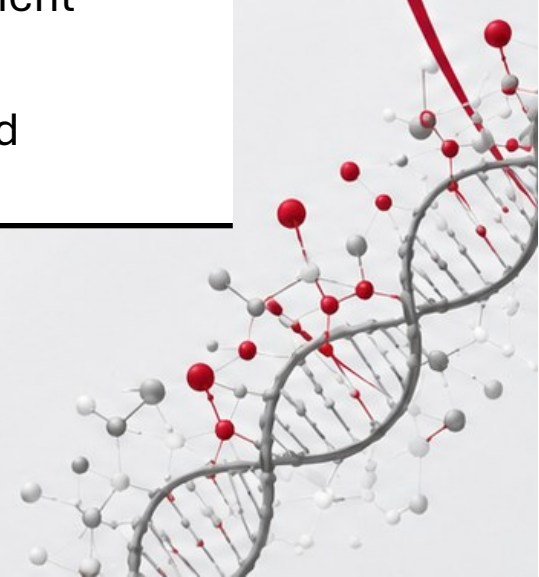
Large

Size determines

- Gradients formation
- Necrotic regions
- Drug/reagent penetration
- Biological response
- Consistency

Tips: What Can Go Wrong?

Common Challenge	Practical Tip
<i>Irregular spheroid formation</i>	Optimize seeding density and plate geometry
<i>Variable spheroid size</i>	Ensure consistent cell seeding and culture conditions
<i>Spheroid loss during medium exchange</i>	Pipette gently along the well wall and consider partial medium changes
<i>Inconsistent drug responses</i>	Standardize spheroid size, age and treatment timing
<i>Unreliable assay signals</i>	Validate reagent penetration, cell lysis and potential assay interference

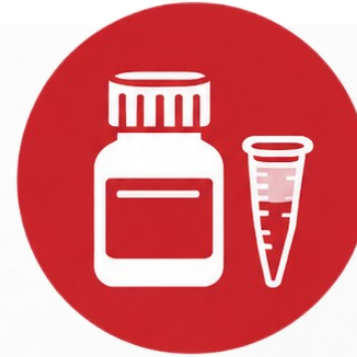


Complex Models Need Better Measurements

- **3D cultures challenges:**

- Harder to penetrate
- Challenging imaging
- Technically demanding
- Cost (vs 2D)

A need for:

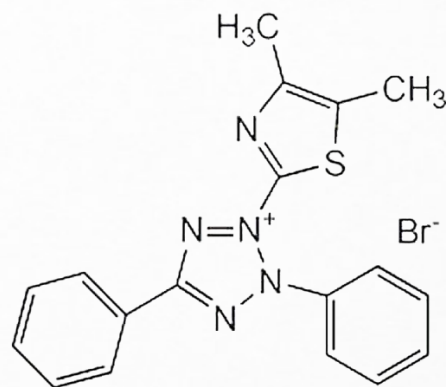


**Robust
reagents**



**Advanced
instrumentation**

A Common Method Isn't Always the Best Choice



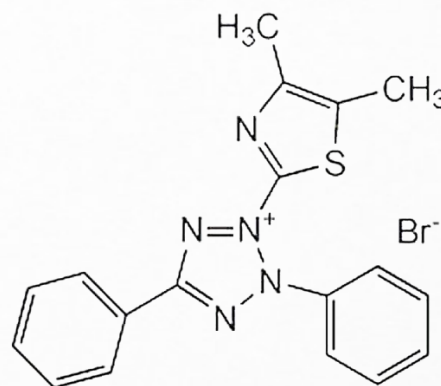
MTT



A Common Method Isn't Always the Best Choice

PROS

- Well-established
- Homogeneous
- Low cost



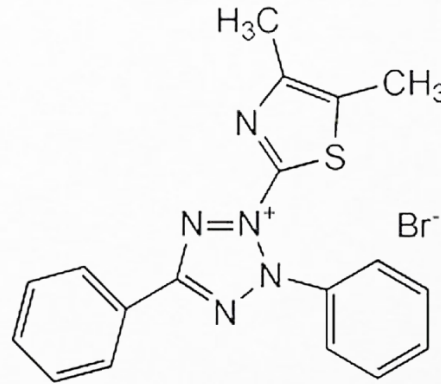
MTT



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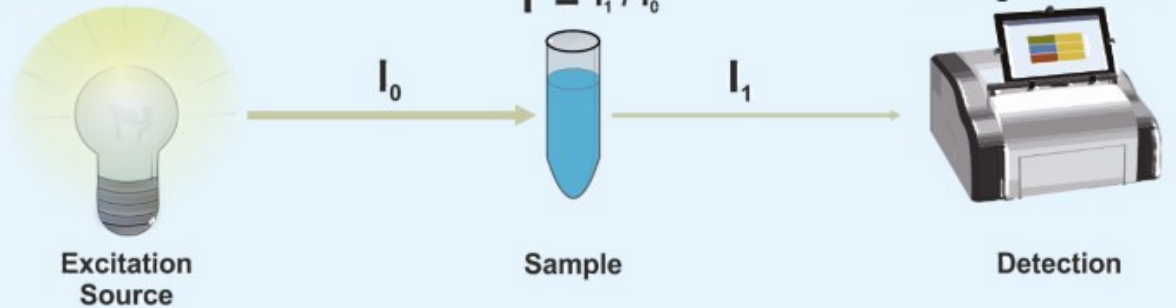
MTT

CONS

- Limited sensitivity
- Chemical interference
- Potential toxicity
- Time consuming
- **Limited penetration**

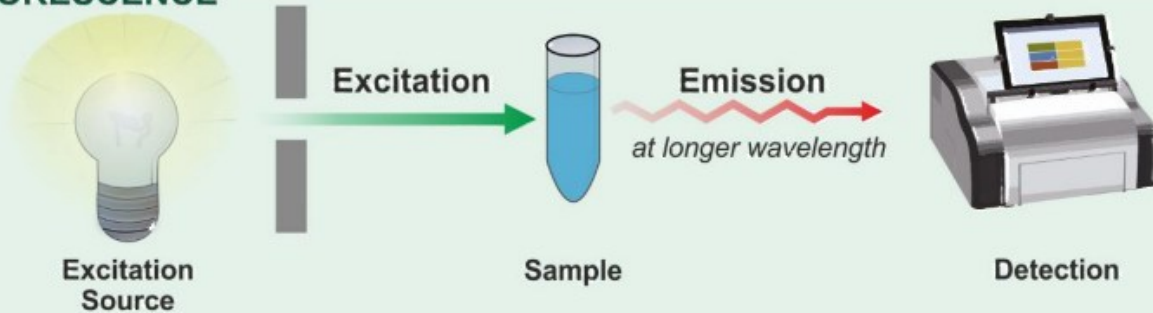
Considering Other Readouts

ABSORBANCE



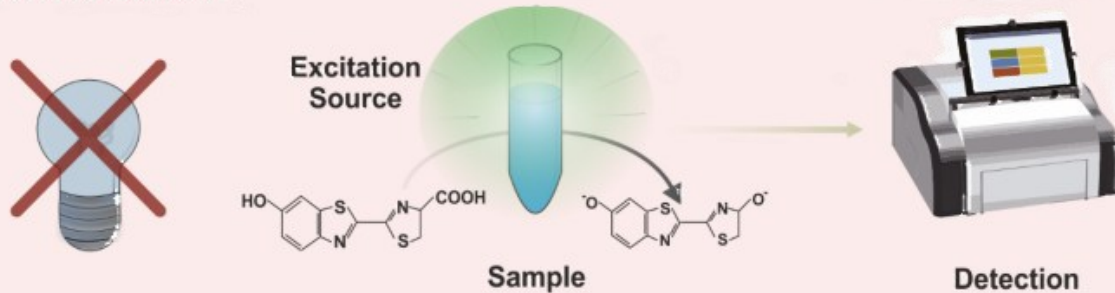
- low sensitivity
- low S/B
- low dynamic range
- no multiplexing
- inexpensive

FLUORESCENCE



- intermediate sensitivity
- intermediate S/B
- intermediate dynamic range; 4 - 5 logs
- multiplexing
- phototoxicity

BIOLUMINESCENCE



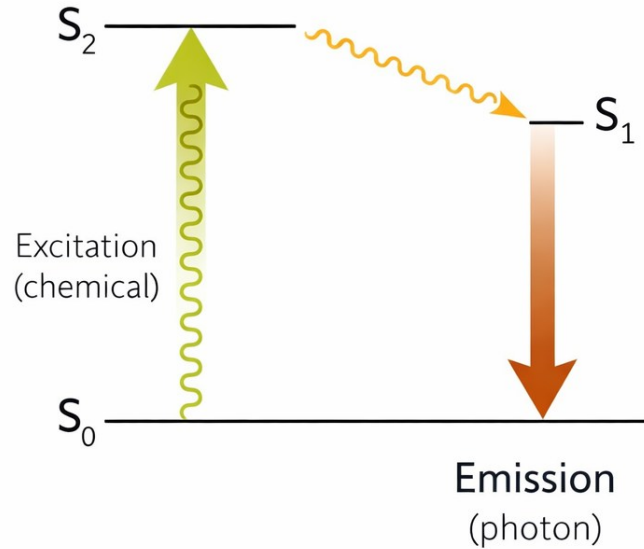
- high sensitivity
- high S/B
- high dynamic range; 8 - 9 logs
- multiplexing
- no phototoxicity



Bioluminescence Leads The Way

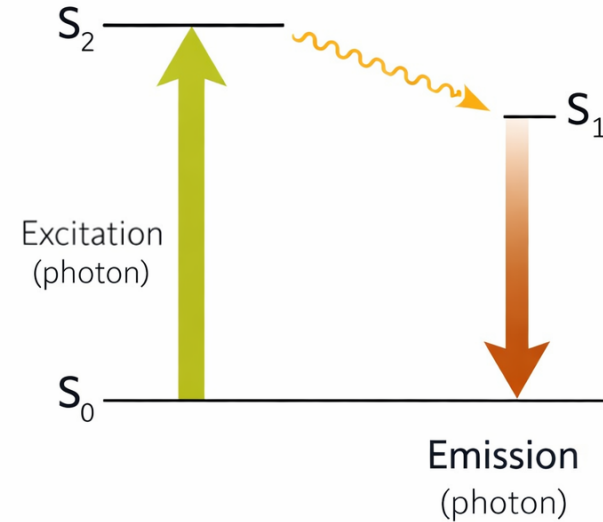
Bioluminescence

Excitation from a chemical reaction



Fluorescence

Excitation from photon



Excitation through chemical reaction

Excitation through light absorption



What's NEW in East Port?

East Port & Promega Seminar

Lukáš Išler

Application Specialist

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Antibodies For EVERY Application



Broad Portfolio

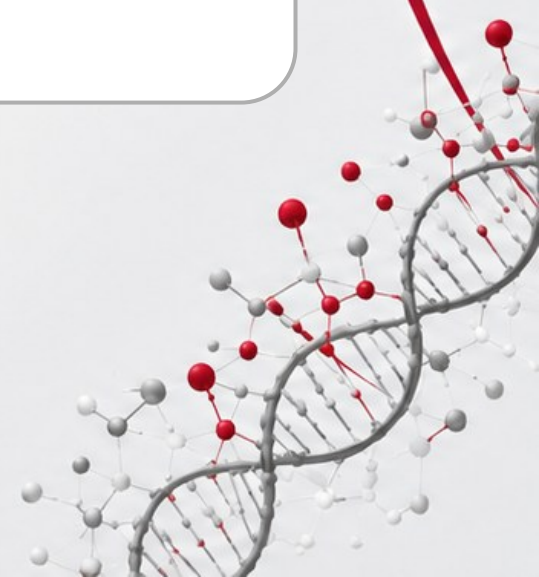
Monoclonal, Polyclonal,
Phospho, Recombinant,
Secondary

Detection Flexibility

Unconjugated, Enzyme-
conjugated, Fluorescent

Multiple Applications

WB, IF/ICC, IHC, Flow
Cytometry, IP



No FBS – A Step to Sustainability



Standard FBS

FBS 100 %



FBS Advanced

FBS 100 %



FBS Xtra

FBS < 50 %



Serexis

FBS 0 %



No FBS – A Step to Sustainability

SEREXIS™ 180

- 1:1 replacement for FBS
- Defined formulation
- Batch consistency
- **NO Lot-to-lot testing**
- Standard 10% concentration



No FBS – A Step to Sustainability

A549

CHO

SEREXIS™ 180

Mouse Th17

Macrophages

HeLa

PG-4

BHK-21

C2C12

MDBK

Primary
Fibroblasts

Vero

HEK293T

Primary
Keratinocytes

Jurkat

Muscle
Cells

MDCK

Mouse Th1

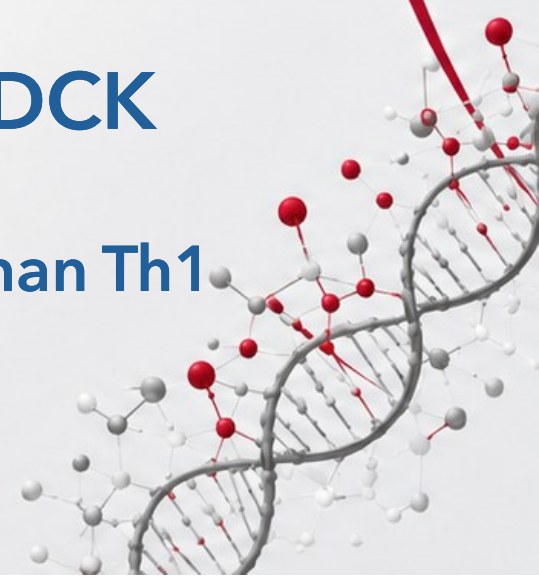
NB324K

Human Th1

L929

Hybridoma

Primary
Immune Cells



TFF Flexi - Automated EV isolation



Check our EV portfolio



Process samples from 10 mL to 60 L



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