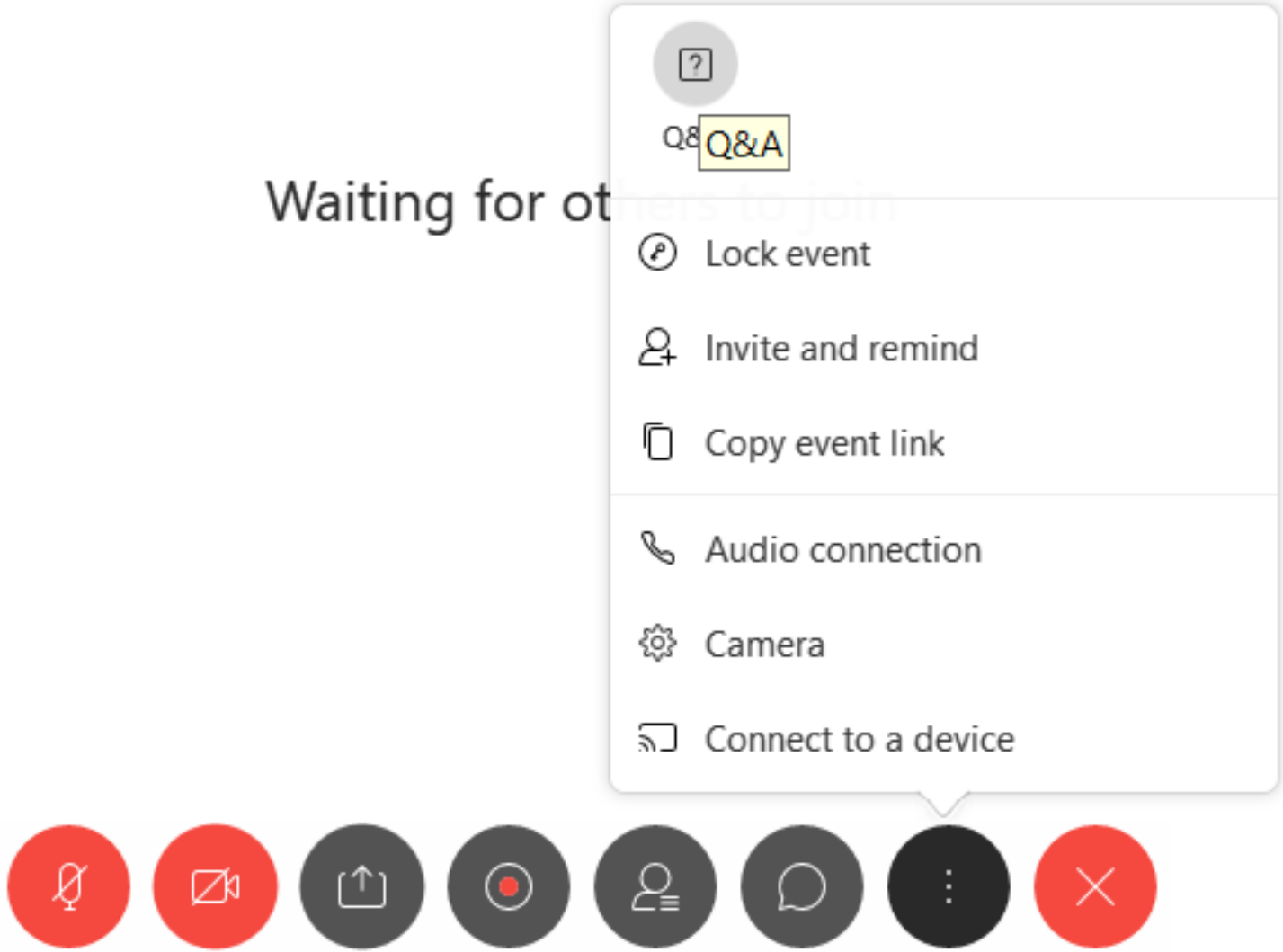
Agenda

17:00	**Welcome**	**Lydia Gan** **Marketing Manager,** **Southeast Asia & Taiwan,** **Life Sciences Solutions,** **Thermo Fisher Scientific**
17:05	**Speeding up your cell therapy workflow:** Next-generation immunoassay	**Ryan Lim** **Field Application Scientist** Immunoassay & Cell Therapy, Thermo Fisher Scientific
17:35	**When setting up qPCR reactions become a matter of minutes**	**Angeline Kan** **Product Manager**, Liquid Handling Solution, Thermo Fisher Scientific
17:45	Q&A	
17:55	Closing	

Q&A

If you have any questions that you would like to pose, please access the Q&A section as shown here



Speeding up your cell therapy workflow: Next generation Immunoassay

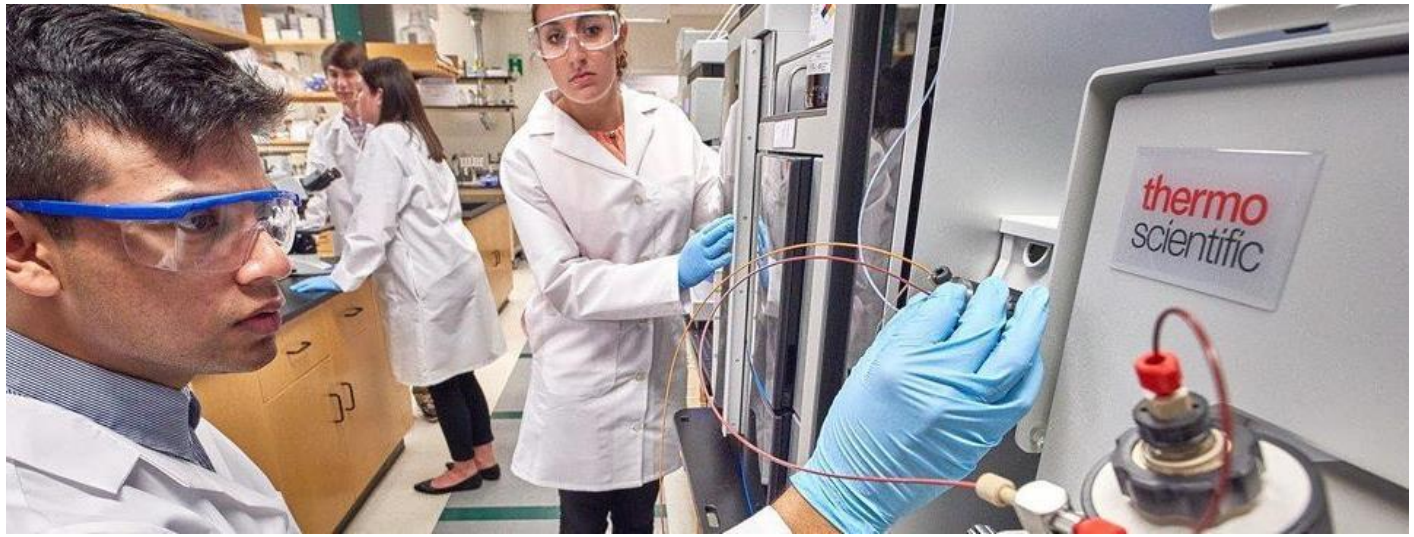
Ryan Lim; PhD

Field Application Scientist (Immunoassay & Cell Therapy)
(Asia Pacific and Japan)

A Unique Customer Value Proposition for Pharma and Biotech

Leading life sciences offering

thermo scientific · applied biosystems · invitrogen · gibco · fisher scientific · unity lab services



Leading contract development and manufacturing organization

fisher clinical services · patheon · brammer bio
part of Thermo Fisher Scientific

Partnering to support our customers—from discovery to molecule to medicine

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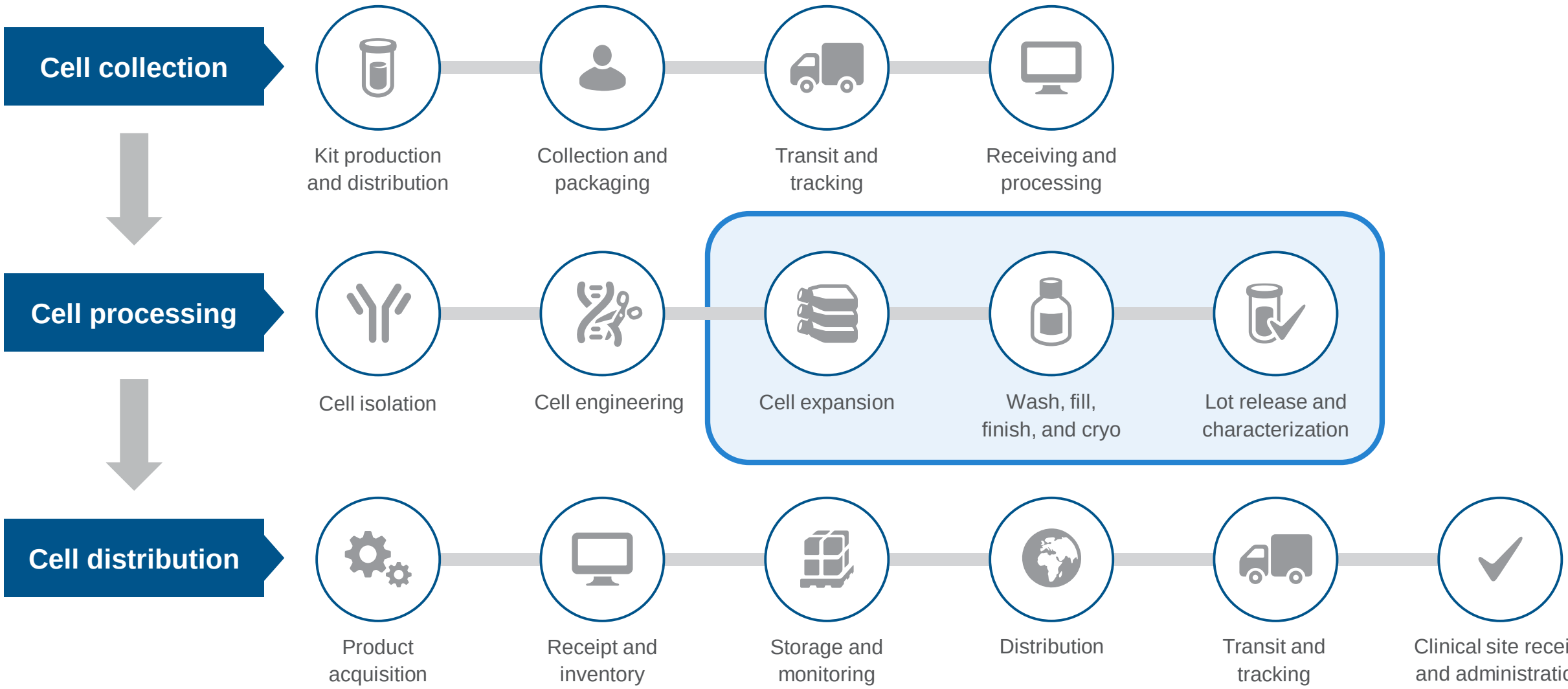
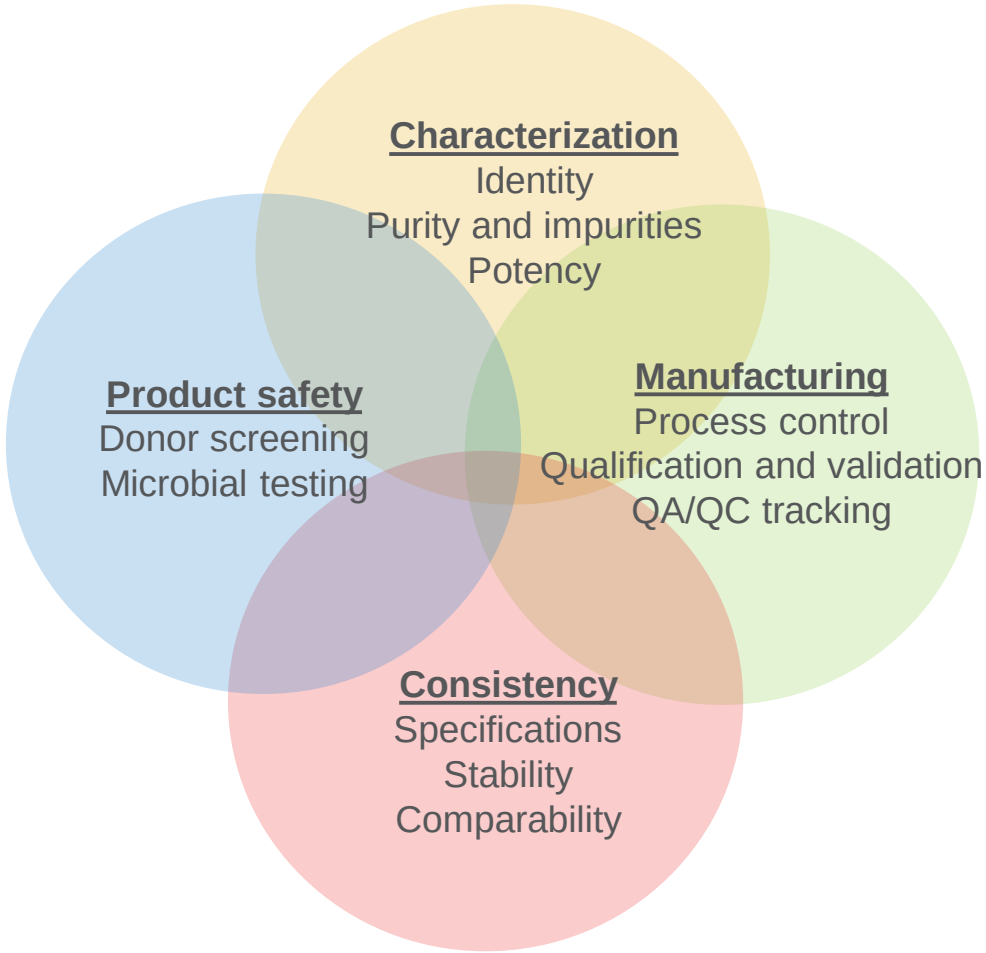
Cell and Gene Therapy Workflows



Safety, Characterization, Manufacturing and Consistency are one



Critical factors that cannot be considered separately

- Product Safety
- Characterization
- Manufacturing
- Consistency

It is not possible to consider one aspect on its own.

What does the US FDA Guidelines recommend regarding ancillary reagents

PRODUCT MANUFACTURING AND CHARACTERIZATION INFORMATION

Ancillary Reagents/ Raw materials

- **Reagents can affect the safety, potency, and purity of the final product, especially by introducing adventitious agents.**
- **Things required to declare for reagent:**
  - Concentration of the reagent at the manufacturing step at which it is used; vendor/supplier; source; reagent quality; COA or cross-reference letters
- **Qualification Program**
  - If not FDA-approved or cleared, additional testing may be needed
  - includes safety testing (sterility, endotoxin, mycoplasma, and adventitious agents), functional analysis, purity testing, and assays (e.g., residual solvent testing) to demonstrate absence of potentially harmful substances.
  - Extent of testing depend on how the specific reagent is used in the manufacturing process.
- **Determination of Removal of Reagents from Final Product**
  - test the final product for residual manufacturing reagents with known or potential toxicities and describe the test procedures you use to detect residual levels of these reagents in the final product

1. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs); Guidance for Industry 1/2020

Our solutions

Cell Therapy Systems (CTS) Products—Designed for Cell Therapy Manufacturing

Gibco™ CTS™ products provide you with a proven choice so you can transition your cell therapy to the clinic with confidence.

- 20 years of cGMP manufacturing cell therapy products
- Extensive safety testing panel and FDA drug master files
- Proven use in FDA-approved cell therapies and over 100 clinical trials

cGMP-compliant manufacturing

- Manufactured in conformity with **cGMP** for medical devices, 21 CFR Part 820
- **FDA-registered** manufacturing site with an ISO 13485–certified quality management system

Testing and documentation

- Traceability documentation—including **Drug Master Files (DMFs)** and certificates of origin
- **Extensive safety testing**—including sterility, endotoxin, adventitious agent, and mycoplasma on applicable products

Proven use

- Used in **FDA-approved CAR-T therapies**[1,2] and the first FDA-approved therapeutic cancer vaccine [3]
- Used in **over 100 clinical trials**

1. Tangying Lily Lu et al. (2016) A rapid cell expansion process for production of engineered the autologous CAR-T cell therapies. *Hum Gene Ther Methods*.
2. http://thermofisher.mediaroom.com/2017-08-30-First-FDA-Approved-Cell-Therapy-for-Leukemia-Utilizes-Thermo-Fisher-Scientifics-CTS-Dynabeads-Technology
3. Madan RA et al. (2011) Sipuleucel-T: harbinger of a new age of therapeutics for prostate cancer. *Expert Rev Vaccines*.

Cell Therapy Media and reagents --- Solutions for your cell type

Workflow Solutions Product Selection Guide

Cell type	T cell	MSC	PSC	NSC	HSC
Media/ supplements	• Gibco™ CTS™ OpTmizer™ T-Cell Expansion SFM • Gibco™ CTS™ AIM-V™ Medium • Gibco™ CTS™ Immune Cell SR	• Gibco™ CTS™ StemPro™ MSC SFM • Gibco™ StemPro™ MSC SFM XenoFree • Gibco™ MesenPRO RS™ Medium	• Gibco™ CTS™ Essential 8™ Medium • Gibco™ CTS™ KnockOut™ SR XenoFree Medium • Gibco™ CTS™ KnockOut™ DMEM	• Gibco™ CTS™ B-27™ Supplement, XenoFree • Gibco™ CTS™ KnockOut™ DMEM/F-12 • Gibco™ CTS™ N-2 Supplement • Gibco™ CultureOne™ Supplement (100X)	• Gibco™ StemPro™-34 SFM
Growth factors/cytokines	• Gibco™ CTS™ IL2 (Interleukin 2) Recombinant Human Protein • Gibco™ CTS™ IL4 (Interleukin 4) Recombinant Human Protein • Gibco™ CTS™ IL7 (Interleukin 7) Recombinant Human Protein • Gibco™ IL15 Recombinant Human Protein • Gibco™ IL21 Recombinant Human Protein • Gibco™ CTS™ GM-CSF Recombinant Human Protein • Gibco™ CTS™ TNF Recombinant Human Protein	• Gibco™ CTS™ FGF-Basic Full Length Recombinant Human Protein • Gibco™ CTS™ TGFB1 Recombinant Human Protein	• Gibco™ CTS™ FGF-Basic Full Length Recombinant Human Protein • Gibco™ CTS™ TGFB1 Recombinant Human Protein • Gibco™ CTS™ SCF (C-Kit Ligand) Recombinant Human Protein • Gibco™ CTS™ FLT3 Ligand Recombinant Human Protein • Gibco™ BMP4 Recombinant Human Protein • Gibco™ EGF Recombinant Human Protein • Gibco™ Activin A Recombinant Human Protein	• Gibco™ CTS™ FGF-Basic Full Length Recombinant Human Protein • Gibco™ EGF Recombinant Human Protein	• Gibco™ CTS™ IL2 (Interleukin 2) Recombinant Human Protein • Gibco™ IL3 Recombinant Human Protein • Gibco™ CTS™ IL4 (Interleukin 4) Recombinant Human Protein • Gibco™ IL5 Recombinant Human Protein • Gibco™ CTS™ IL6 (Interleukin 6) Recombinant Human Protein • Gibco™ CTS™ IL7 (Interleukin 7) Recombinant Human Protein • Gibco™ CTS™ SCF (C-Kit Ligand) Recombinant Human Protein • Gibco™ CTS™ GM-CSF Recombinant Human Protein • Gibco™ CTS™ FLT3 Ligand Recombinant Human Protein • Gibco™ TPO (Thrombopoietin), Recombinant Human Protein • Gibco™ M-CSF Recombinant Human Protein
Extracellular matrix	• NA	• Gibco™ CTS™ CELLstart™ Substrate	• Gibco™ CTS™ Vitronectin (VTN-N) Recombinant Human Protein • Gibco™ CTS™ CELLstart™ Substrate • Gibco™ rhLaminin-521	• Gibco™ CTS™ CELLstart™ Substrate	• NA
Reagents	• Gibco™ CTS™ Dynabeads™ CD3/CD28 • Gibco™ CTS™ DPBS • Gibco™ CTS™ GlutaMAX™-I Supplement • Gibco™ L-Glutamine • Gibco™ LV-MAX™ Lentiviral Production System	• Gibco™ L-Glutamine • Gibco™ StemPro™ Osteogenesis Differentiation Kit • Gibco™ StemPro™ Chondrogenesis Differentiation Kit • Gibco™ StemPro™ Adipogenesis Differentiation Kit • Gibco™ CTS™ DPBS • Gibco™ CTS™ GlutaMAX™-I Supplement • Gibco™ CTS™ TrypLE™ Select Enzyme • Invitrogen™ Lipofectamine™ Stem Transfection Reagent	• Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit • Gibco™ PSC Cardiomyocyte Differentiation Kit • Gibco™ PSC Dopaminergic Neuron Differentiation Kit • Gibco™ PSC Definitive Endoderm Induction Kit • Gibco™ CTS™ DPBS • Gibco™ CTS™ GlutaMAX™-I Supplement • Gibco™ CTS™ TrypLE™ Select Enzyme • Invitrogen™ Lipofectamine™ Stem Transfection Reagent	• Gibco™ CTS™ DPBS • Gibco™ CTS™ GlutaMAX™-I Supplement • Gibco™ CTS™ TrypLE™ Select Enzyme • Invitrogen™ Lipofectamine™ Stem Transfection Reagent	• Invitrogen™ Dynabeads™ CD34 Positive Isolation Kit • Gibco™ CTS™ DPBS • Gibco™ CTS™ GlutaMAX™-I Supplement
Preservation	• Gibco™ CTS™ Synth-a-Freeze™ Medium	• Gibco™ CTS™ Synth-a-Freeze™ Medium	• Gibco™ CTS™ Synth-a-Freeze™ Medium • Gibco™ PSC Cryopreservation Kit	• Gibco™ CTS™ Synth-a-Freeze™ Medium	• Gibco™ CTS™ Synth-a-Freeze™ Medium

Regarding efficacy: Potency assay

What is potency

Purpose for Potency

- Potency measurements are a necessary part of product characterization testing, comparability studies, and stability protocols, which are used to establish that a consistently manufactured product is administered during all phases of clinical investigation.
- No single test can predict clinical efficacy, hence demonstrate by “substantial evidence”
- Need to develop a release assay to measure your product’s potency.

What should be measured for Potency

Product Characterization

- Recommend that you measure a wide range of product attributes in addition to the tests used for routine lot release

Mechanism of Action

- Ideally have MOA, but due to complexity, there is some leeway, but all attempt must first be made

1. FDA Potency Tests for Cellular and Gene Therapy Products (Final Guidance for Industry): JANUARY 2011
2. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. **Cytotherapy. 2016 Feb;18(2):151-9**

US FDA Guidelines for Potency

Potency Assay Development

- Recommended to start early as it:
  - Demonstrate product activity, quality and consistency throughout product development
  - Generate a collection of data to support specifications for lot release
  - Provide a basis for assessing manufacturing changes
  - Evaluate product stability
  - Recognize technical problems or reasons a different assay might be preferable

- If you do **not provide sufficient assurance** of potency of product lots to be used in your pivotal trial(s), your trial may be considered "deficient in design to meet its stated objectives" and may be placed on clinical hold (21 CFR 312.42(b)(2)(ii)).

Potency Assay Validation

- Product meets prescribed requirements of potency (21 CFR 601.2), which requires that you validate your potency assay (21 CFR 211.165(e)).

Accuracy

Specificity

Linearity and Range

System Suitability

Robustness

Precision (Repeatability, intermediate precision and reproducibility)

1. FDA Potency Tests for Cellular and Gene Therapy Products (Final Guidance for Industry): JANUARY 2011

Cell Therapy cell type and the cytokine connection

MSC

MSC Immunomodulatory Potential

- By affecting B-cells, Dendritic cells, Monocytes/macrophages, NK cells and T-cells
- Effect have been attributed to its secretion of cytokine

Table 1 Cytokines secreted by mesenchymal stem cells and the corresponding target cells [2]

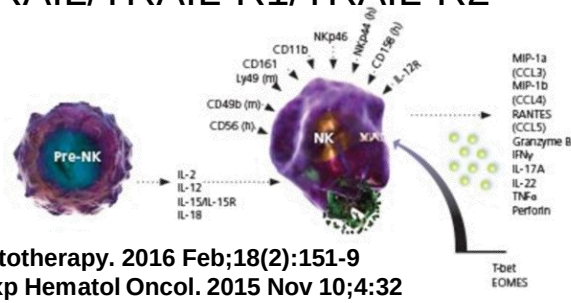
Cytokines secreted by MSCs	Target cells
IL-10	Mph, Neu, DCs, Th1, Tregs, Tr1, tumor cells
IL-6	Neu, Mo, DCs, B, Th2, Tregs, Th17, CD8+FoxP3+
TGFβ	Mph, NK, DCs, B, T, Tregs
Chemokines	Neu, Mo, NK, Eo, Baso, DCs, Ly
CCL-2/MCP-1	Mph, EC, PL, Th2, Th17
CCL-5/RANTES	Neu, Mo, DCs, Th1, Tregs, CD8+FoxP3+
IDO	Mo, DCs, B, T, Tregs
VEGF	DCs, EC, Th1, Th17, Tregs
ICAM	T, MSCs
PGE2	Mph, Mo, NK, DCs, T, Tr1

NK

NK Cell	Cytokine-Induced NK (CIK)-NKT	Cytokines T Lymphocyte (CTL)

Critical Factors for efficacy to study

- Release of cytotoxic granules to mediate cell apoptosis
  - Perforin; granzyme
- Secreted cytokines and chemokines
  - IFN-gamma, TNF-alpha, IL-4, IL-5, IL-10, IL-17A, and IL-22
- Death-Ligand/death receptor-mediated cell apoptosis
  - TNF/TNF-R1; FasL/Fas; TRAIL/TRAIL-R1/TRAIL-R2



1. Chapter 20 - Cytotoxic T Cells , **Immunology for Pharmacy 2012 Pages 162-168**
2. Secretion of immunoregulatory cytokines by mesenchymal stem cells **World J Stem Cells. 2014 Nov 26; 6(5): 552–570.**
3. Immunomodulation by Mesenchymal Stem Cells (MSCs): Mechanisms of Action of Living, Apoptotic, and Dead MSCs **Front Immunol. 2019 Jun 4;10:1191**
4. Cell-based immunotherapy with cytokine-induced killer (CIK) cells: From preparation and testing to clinical application. **Hum Vaccin Immunother. 2017 Jun; 13(6): 1379–1387.**
5. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. **Cytotherapy. 2016 Feb;18(2):151-9**
6. The combination of dendritic cells-cytotoxic T lymphocytes/cytokine-induced killer (DC-CTL/CIK) therapy exerts immune and clinical responses in patients with malignant tumors. **Exp Hematol Oncol. 2015 Nov 10;4:32**

Examples of reviewing cytokine signatures for CART-T immunotherapy

Sci Transl Med. 2011 Aug 10;3(95):95ra73. doi: 10.1126/scitranslmed.3002842.

T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia.

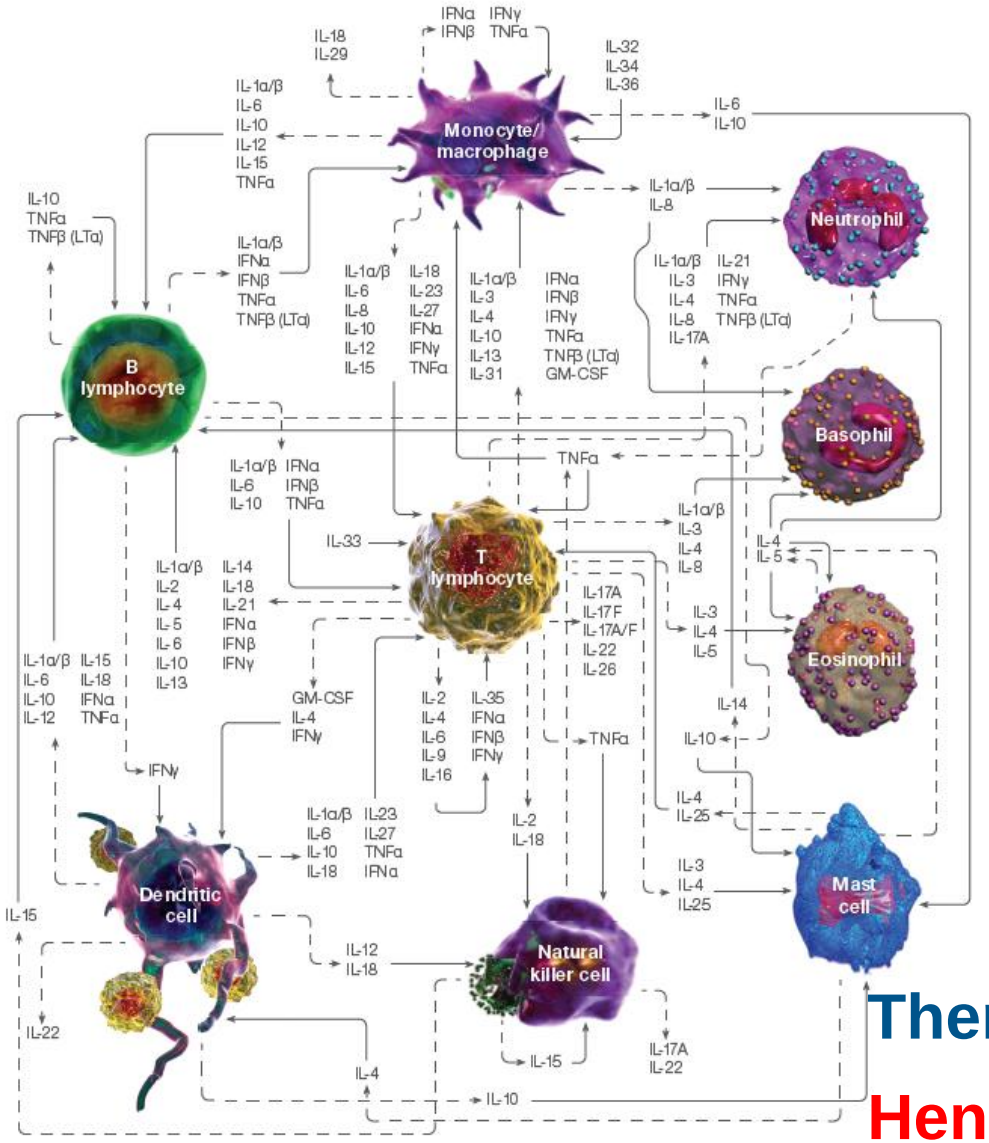
Kalos M[1], Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH.

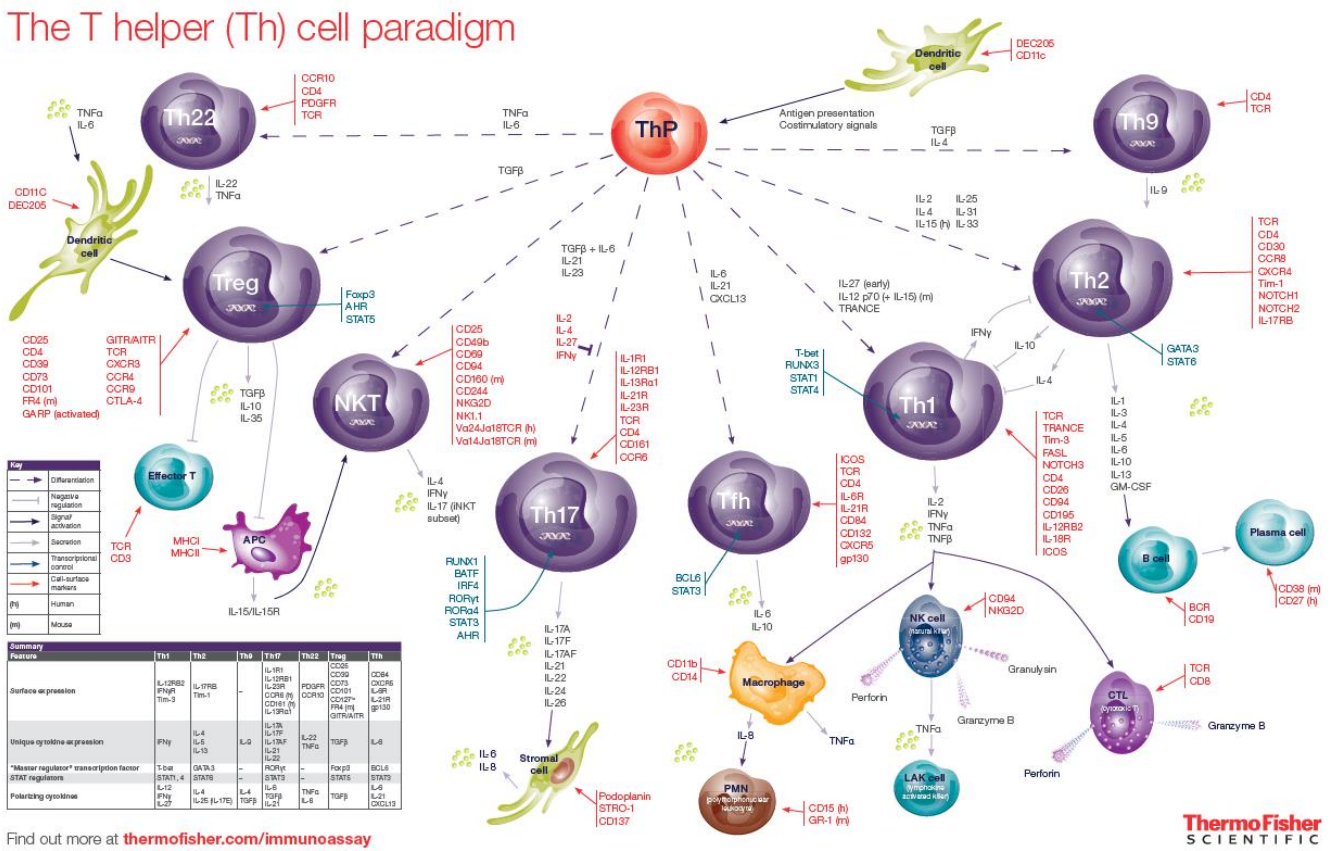
This study investigated 30 different cytokines using a 30-Plex panel over multiple timepoints. 11 cytokines showed significant changes post-transfusion.

Excerpt from YESCARTA™ (axicabtagene ciloleucel) Package Insert

"After YESCARTA infusion, pharmacodynamic responses were evaluated over a 4-week interval by measuring transient elevation of cytokines, chemokines and other molecules in blood. Levels of cytokines and chemokines such as IL-6, IL-8, IL-10, IL-15, TNF-α, IFN-γ, and sIL2Rα were analyzed. Peak elevation was observed within the first 14 days after infusion, and levels generally returned to baseline within 28 days. "

Cytokine Network





There are more than just one cytokine in play

Hence, conventional methods would not be effective

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Our Solutions

Direct and consistent: (Improving Gold-standard technology)

Challenges of …

Consistent Data

- Variability due to multiple steps
- Need direct method

Time-to-results

- Long testing cycles delays lot release

Innovation: Instant™ ELISA Kit

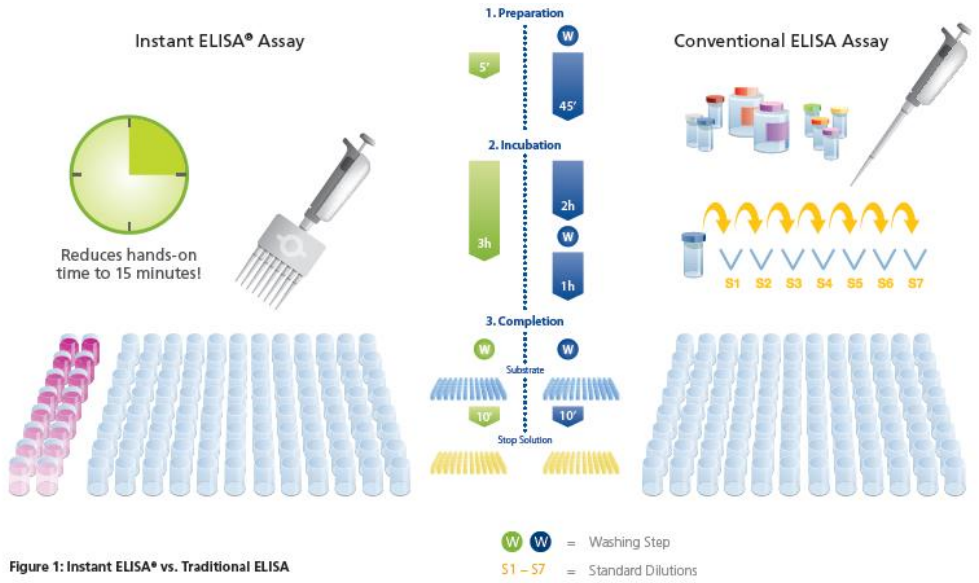

Figure 1: Instant ELISA® vs. Traditional ELISA

- **Increase consistency of data by reducing human error**
- **Time saving due to single wash**

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Instant™ ELISA Kit

17 Steps for Conventional ELISA	
01	Washing of coated plate
02	Reconstitution of standard protein
03	Addition of sample diluent to standard wells
04	Titration of standard curve
05	Addition of sample diluent
06	Sample addition
07	Dilution of biotin conjugate
08	Addition of biotin conjugate
09	Incubation
10	Preparation of streptavidin-HRP
11	Washing step
12	Addition of streptavidin-HRP
13	Incubation
14	Washing step
15	Addition of TMB substrate
16	Addition of stop solution
17	Calculation of results

7 Steps for Instant ELISA	
01	Rehydration of plate
02	Sample addition
03	Incubation
04	Washing step
05	Addition of TMB substrate
06	Addition of stop solution
07	Calculation of results

Greater consistency in shorter time

- **Standard curve is pre-made in lyophilized form.**
- **Single wash step only**
- **Antibodies are pre-mixed**

Lesser hands-on time = Lesser chance of error

Solving Challenges — (The solution we want)

Challenges of …

Product complexity

- Requires multiple targets
- Validation required

Time-to-results

- Long testing cycles delays lot release

Trust in data

- Accuracy, precision, sensitivity, range and robustness
- Simple and straight forward

Innovation: ProcartaPlex (Multiplex Immunoassay)

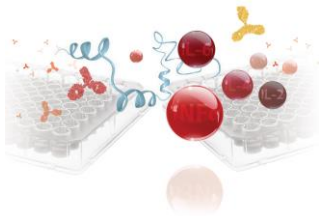
Specific like a sandwich ELISA (antibody pair)

Analysis of up to 80 analytes (50 with the Luminex® MAGPIX® system) in a single sample

Low sample volume needed—25 µL for plasma or serum, or 50 µL for cell culture supernatant

Save cost— price per target/sample decrease with more protein targets

Save time— run equivalent of multiple ELISAs in 1 day (4-5hrs)



Secreted proteins simultaneously measured using ProcartaPlex Cytokine Assay.

Bead-Based Assay Technology

Invitrogen™ ProcartaPlex™ immunoassays apply Luminex® xMAP® technology

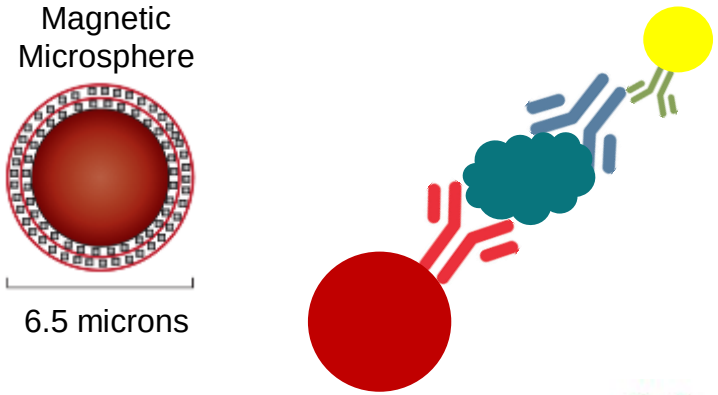
80 sets of superparamagnetic 6.5 µm microspheres

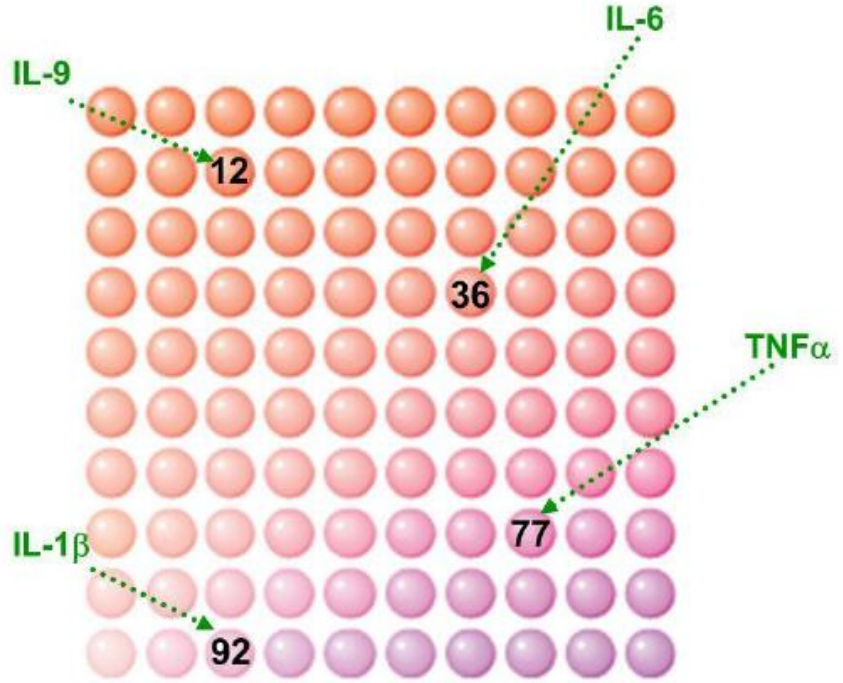
Each bead set is associated with a specific analyte—in essence, giving rise to multiple different immunoassays in a single well.

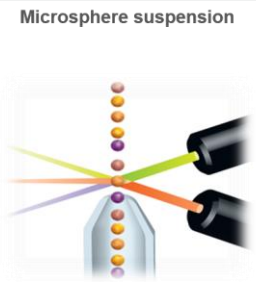
Beads are dyed using a particular bead-to-dye ratio that gives each a particular signature.

Each bead color is assigned to an analyte.

A different target can be bound to each unique bead type.

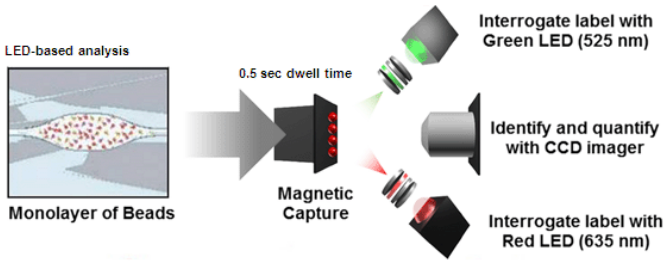






Detects intensity of phycoerythrin (PE) with higher **quantitation**

Detects bead with higher **specificity (analyte)**



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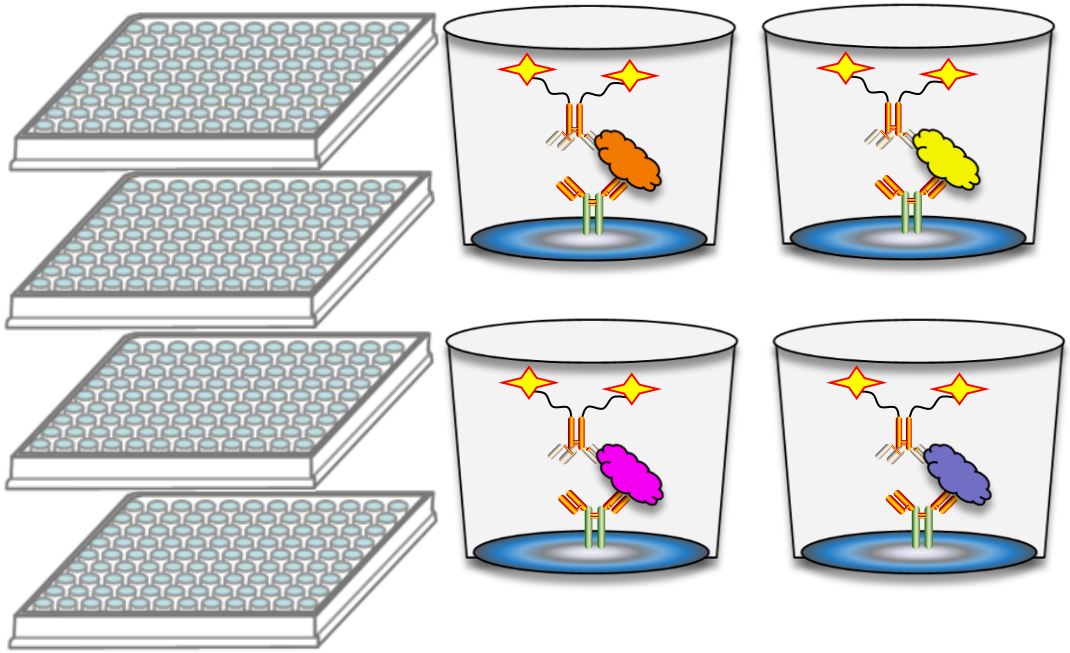
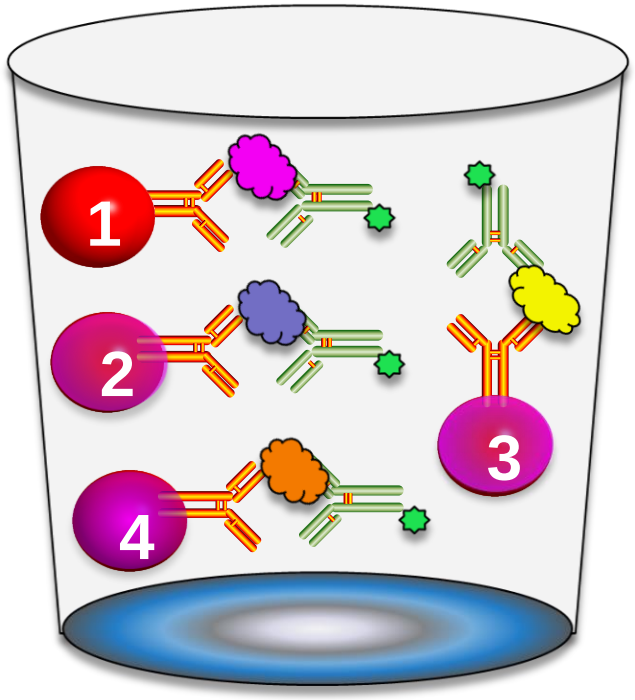
Procartaplex VS ELISA

IL-8 IFN-γ TNF-α GM-CSF

Traditional ELISA



Procartaplex



25-50uL sample/well

- Save Sample
- Save Money
- Save Time
- Increase accuracy

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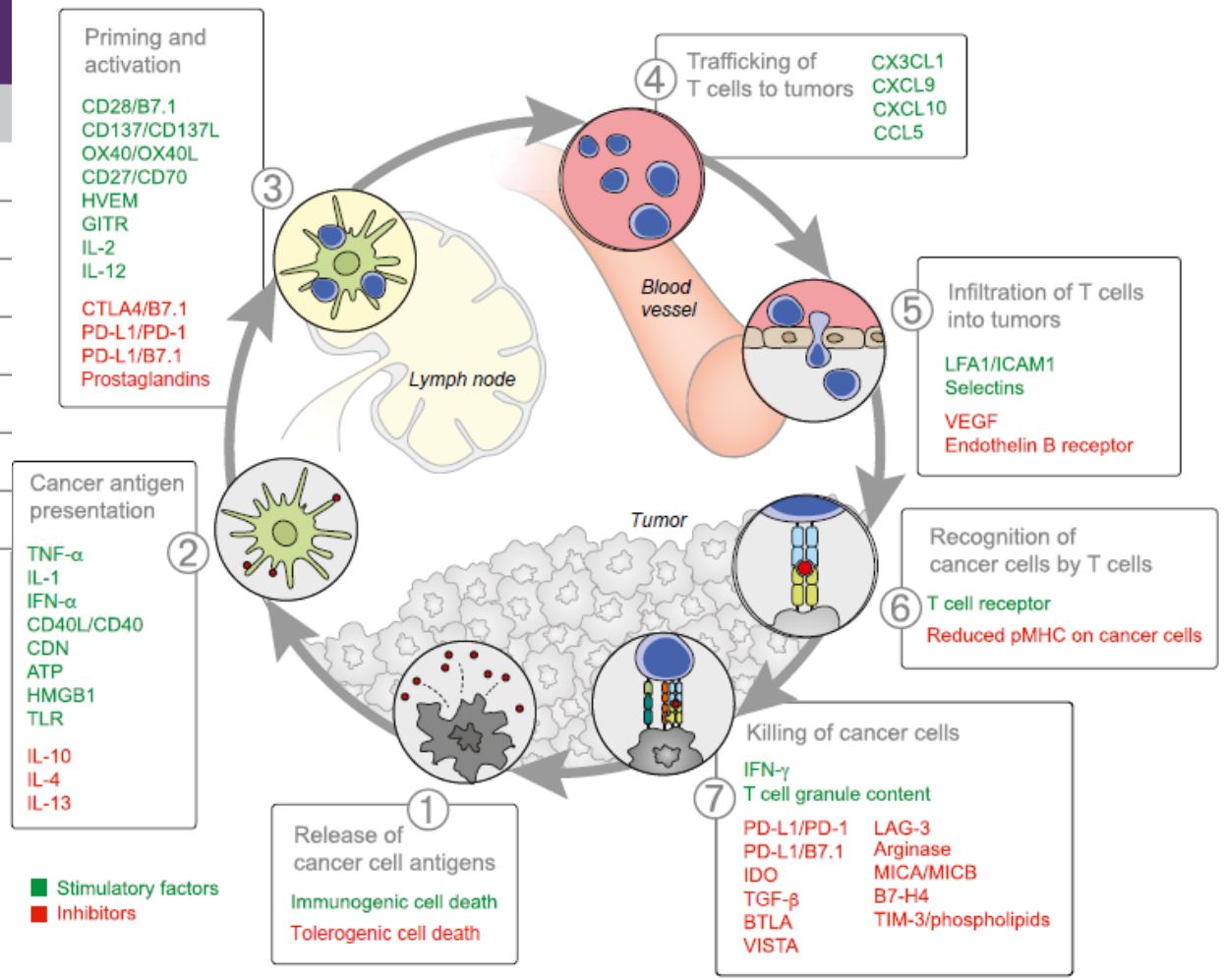
Immuno-panels

Immuno-Oncology Checkpoint 14-Plex Human ProcartaPlex Panel 1 Cat. No. EPX14A-15803-901	
Preconfigured targets	
CD28	PD-1
CD80 (B7-1)	PD-L1
CD137	PD-L2
CD27	IDO
HVEM	BTLA
GITR	LAG-3
CTLA4	TIM-3



Immuno-Oncology Checkpoint 14-Plex Human ProcartaPlex Panel 2 Cat. No. EPX140-15815-901	
Preconfigured targets	
Arginase-1	Siglec 7
CD112 (nectin-2)	Siglec 9
CD155 (PVR)	sMICA
CD73 (NT5E)	sMICB
CD96 (tactile)	sULBP 1
E-cadherin	sULBP 3
Perforin	sULBP 4

ProcartaPlex Custom Panel Configurator and Selection Tool

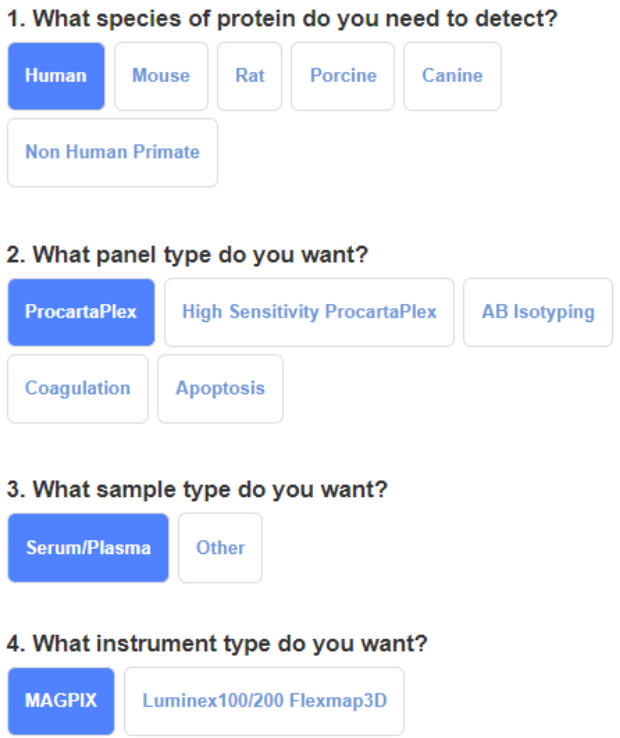
www.thermofisher.com/order/luminex/ The panel is created by single click buttons and will turn blue when selected.

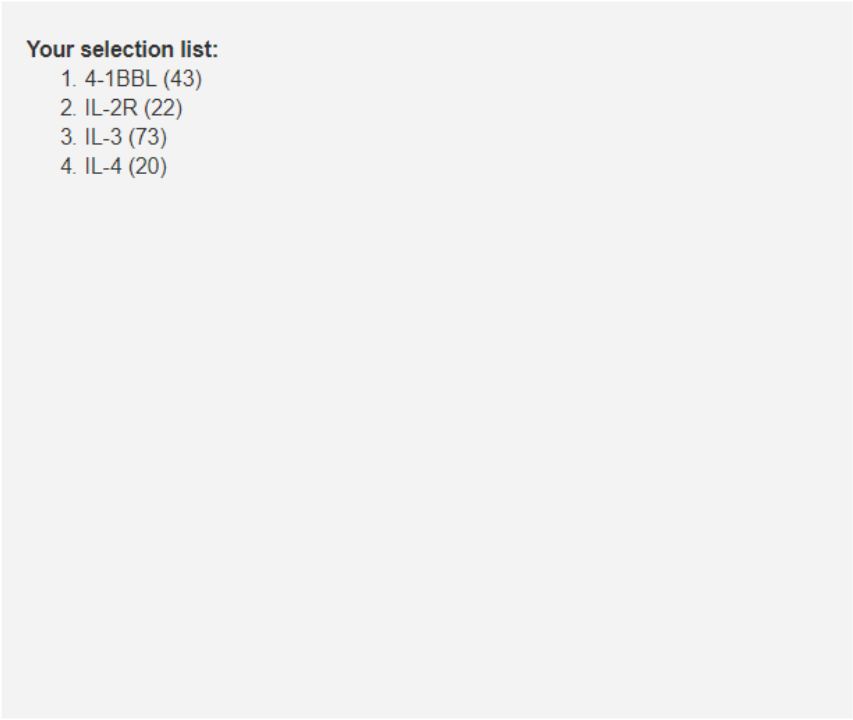
Species →

Assay →

Sample →

Instrument →



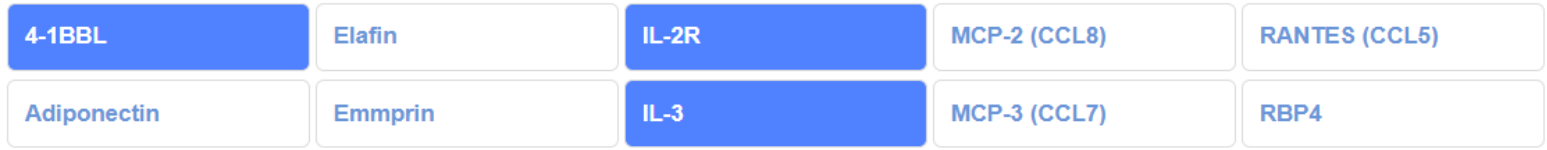


Next Step

Analyte selection

Blue: analyte selected

Red: analyte not compatible



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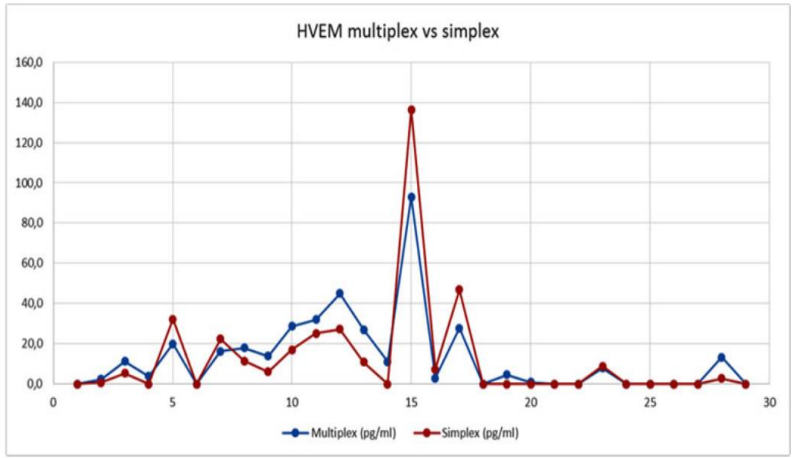
ProcartaPlex: Analyte specificity and Scalability with Consistency

Cross-reactivity testing of analytes in preconfigured panels

Each individual recombinant protein spiked in S1 concentration.	Cross-reactivity in %													
	BTLA	GITR	HVEM	IDO	LAG-3	PD-1	PD-L1	PD-L2	TIM-3	CD28	CD80	CD137	CD27	CD152
BTLA	--	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
GITR	0,0%	--	0,0%	0,0%	0,0%	0,0%	2,9%	0,0%	4,4%	0,0%	0,0%	0,0%	0,0%	0,0%
HVEM	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
IDO	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
LAG-3	0,2%	1,3%	0,3%	0,9%	--	0,8%	1,8%	2,7%	9,8%	0,8%	0,2%	0,8%	0,9%	1,3%
PD-1	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
PD-L1	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
PD-L2	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
TIM-3	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%	0,0%
CD28	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%	0,0%
CD80	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%	0,0%
CD137	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%	0,0%
CD27	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--	0,0%
CD152	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	--

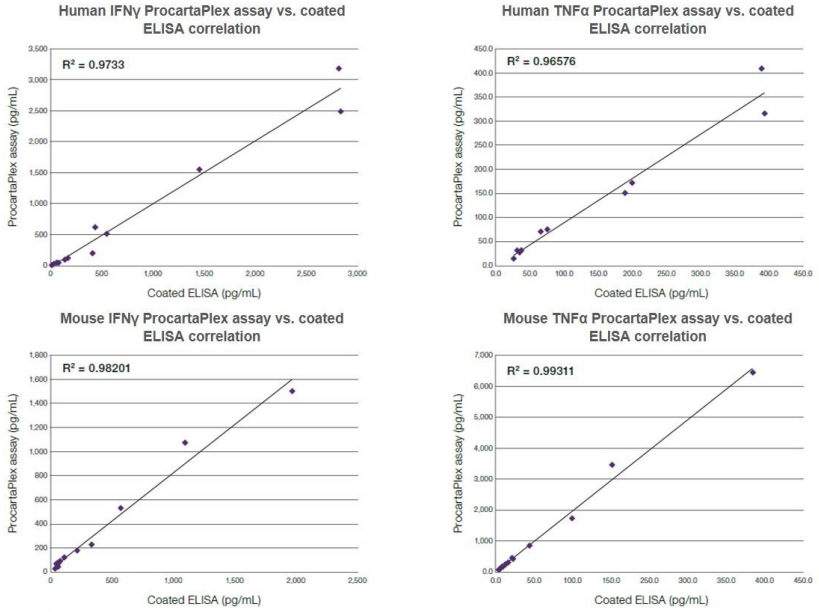
- Measuring a matrix spiked with known concentrations of a variety of different but related analytes
- Measuring a single recombinant protein within a mixture of different bead conjugates

Scalability with consistency



- We use the same antibodies between bigger panels and smaller panel
- Allow for translation of data

Data mappable to ELISA



- Switch easily from ProcartaPlex assays to ELISAs with reliable results
- Most of our ProcartaPlex assays use the same antibody pairs as our ELISAs`

Introducing ProQuantum Immunoassays – a Leap in Next Gen Innovation

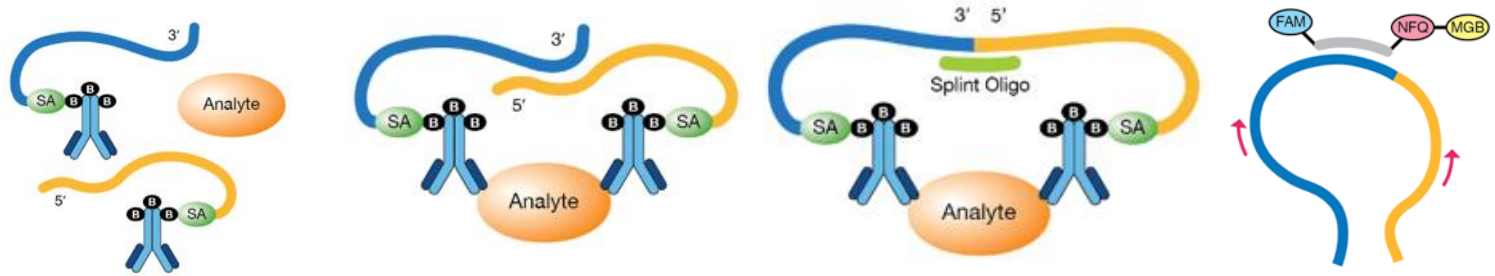
A simple, yet powerful platform for target-specific protein quantitation

High-sensitivity	Detect low levels of proteins (fg/ml) with greater sensitivity than traditional methods like ELISA
Small sample consumption	Use 2-5 µL of sample (e.g., 2 µL vs 75 µL for triplicate wells with other methods)
Fast, easy workflow	Homogeneous (no wash) assay can provide sample to answer in 2 hours
Broad dynamic range	Can reach 5 logs, minimizing sample dilutions to ensure falling within the range
No proprietary instrument to purchase	Runs on any real-time PCR instrument
Includes intuitive cloud-based software	For robust data analysis and statistical group wise comparison

Specificity of matched antibody pairs

+

Sensitivity and broad dynamic range of qPCR



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A New Generation Immunoassay Platform with an Intuitive Interface and Analytics

Standard curve wizard

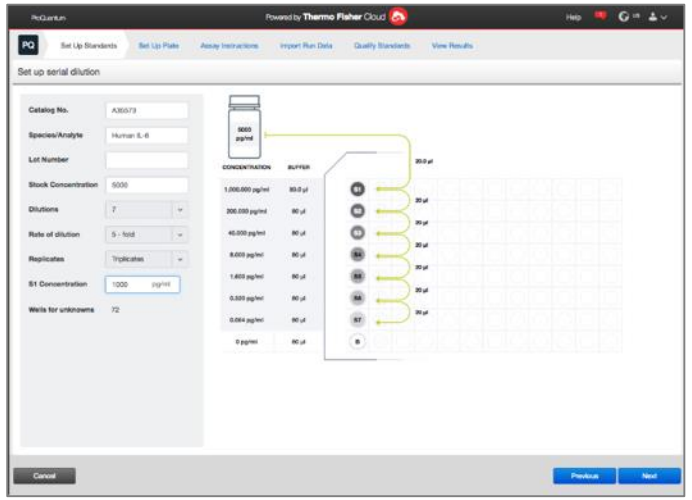
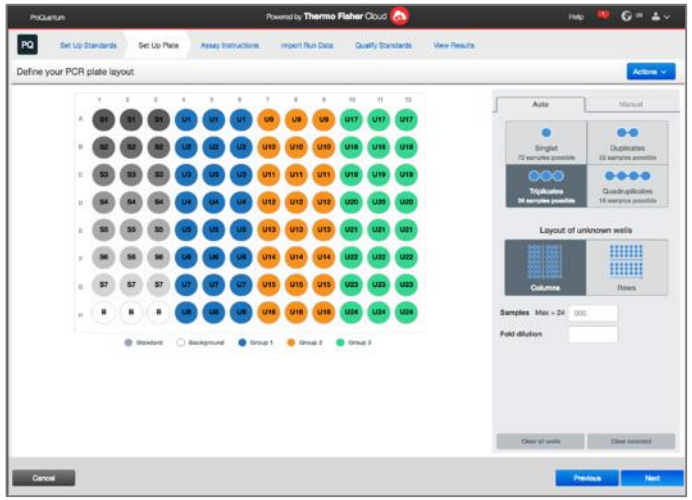
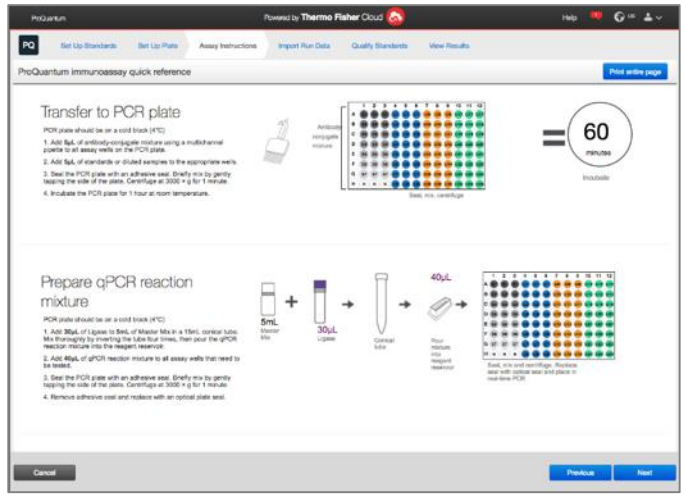


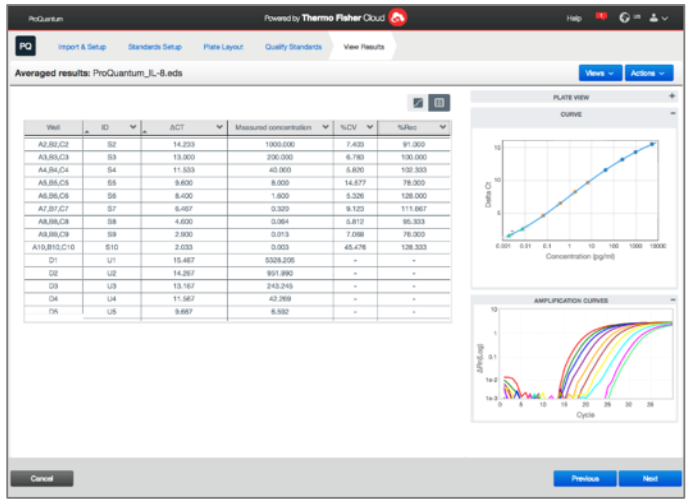
Plate setup wizard



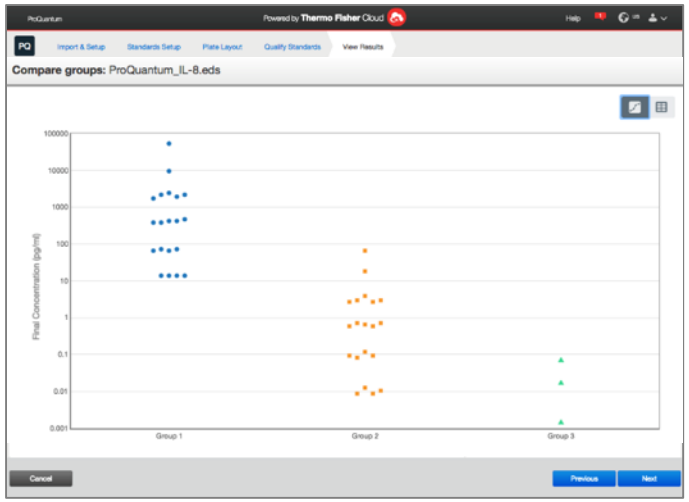
Customized lab instructions



Sample analyte concentrations



Group wise statistics

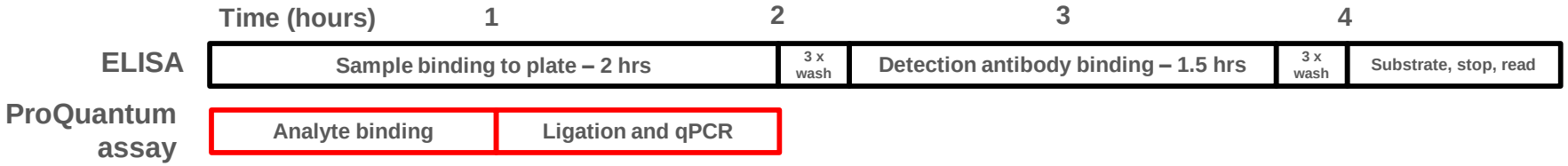


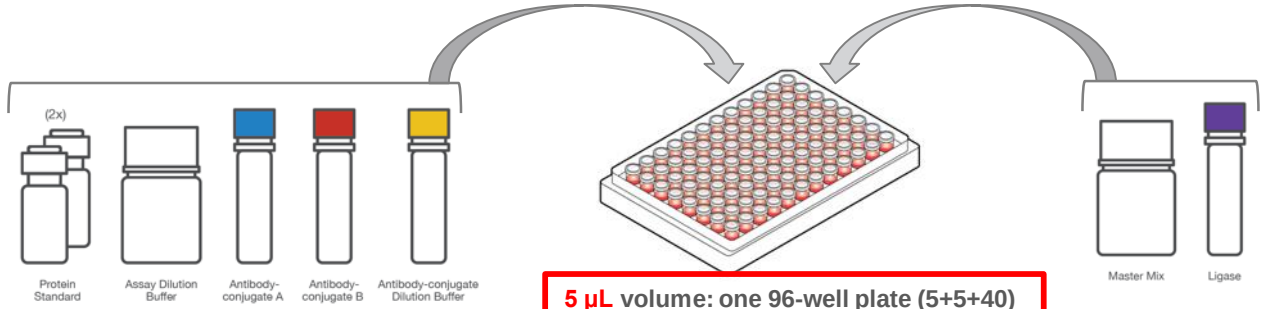
Powered by **Thermo Fisher** Cloud

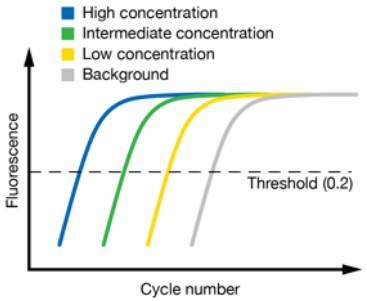
- Guided assay setup is lab notebook / e-notebook friendly
- Export data GraphPad Prism software
- Instrument run monitoring app
- Compatible with **ALL** real-time PCR instruments
- Linked to online training resources

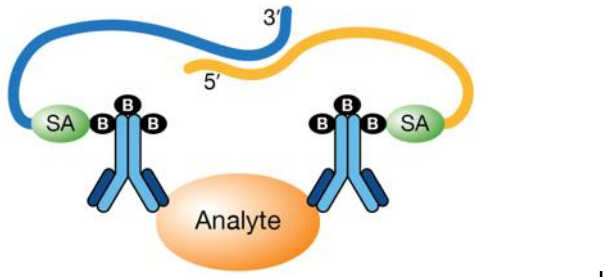
ThermoFisher SCIENTIFIC

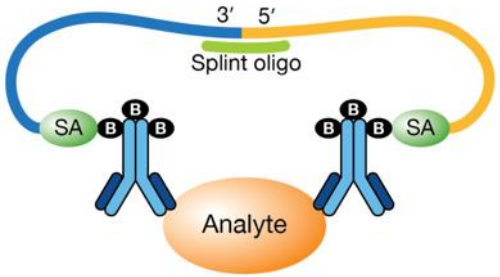
ProQuantum High-sensitivity Immunoassays are Rapid and Robust

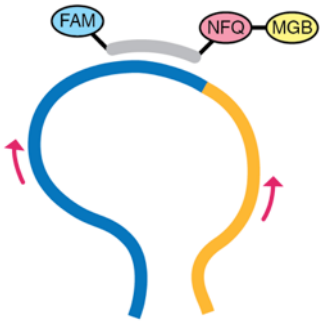












Step 1: analyte binding

- 5 µL sample + 5 µL antibodies
- 1 hr incubation at room temperature

Step 2: ligation and qPCR readout

- Add 40 µL ligase and qPCR master mix
- In-instrument ligation and 40 cycles qPCR

Transfer .EDS file or .CSV file with Ct values in plate map orientation

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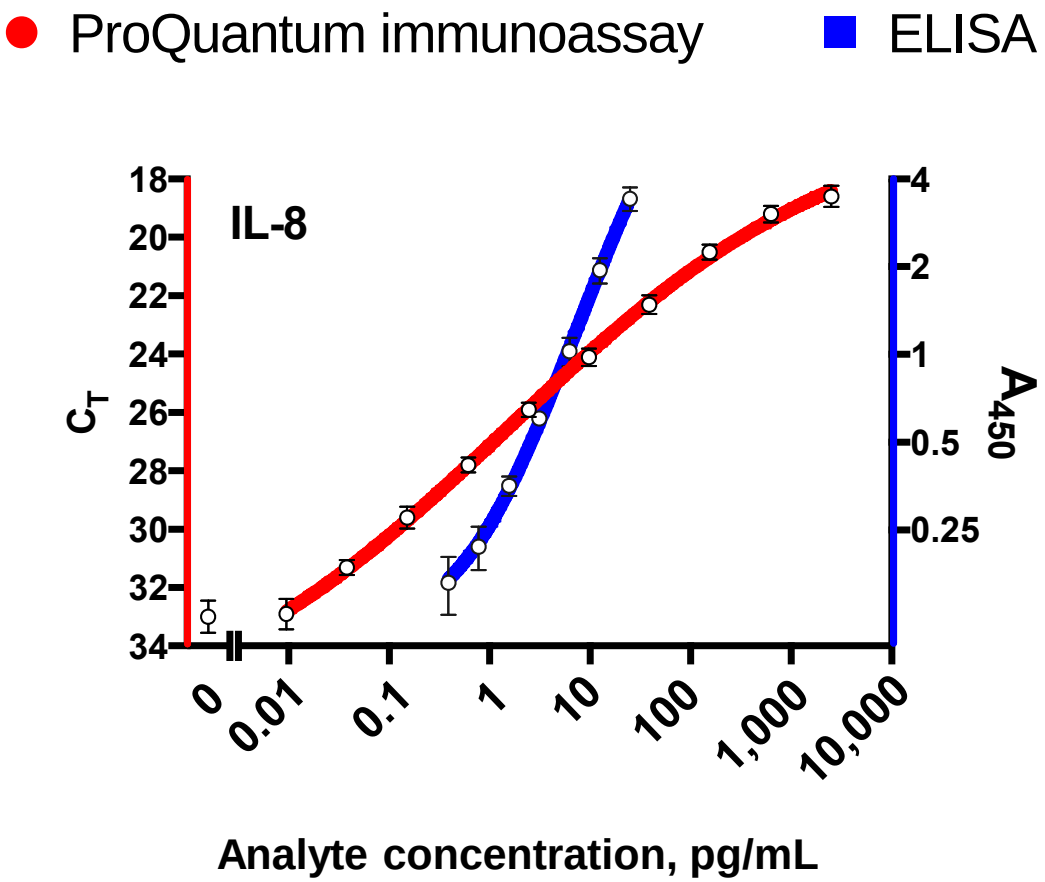
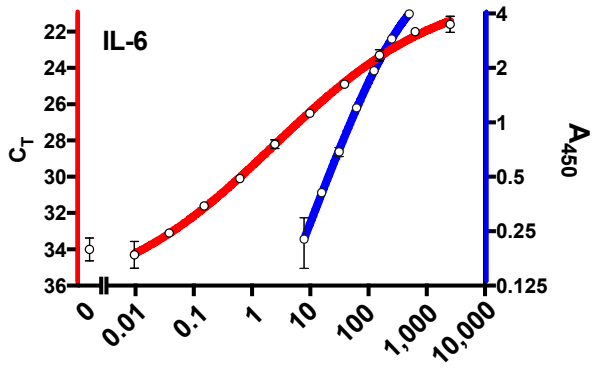
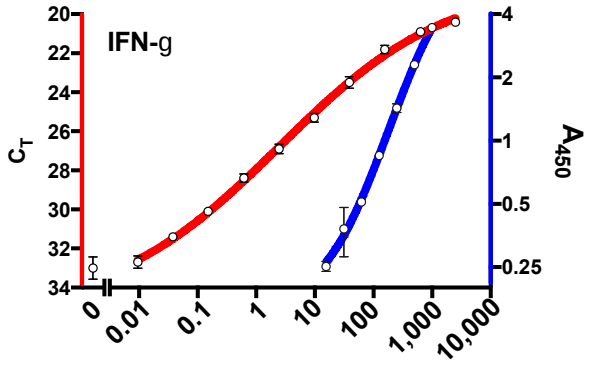
Superior Sensitivity and Dynamic Range Using Only a Fraction of Sample Volume







Assay characteristics and performance include:

- ✓ Serum spike-and-recovery: 80% - 120%
- ✓ Plasma spike-and-recovery: 70% - 130%
- ✓ Parallelism and linearity of dilution: >0.9
- ✓ Protein standard calibration to NIBSC or leading ELISA
- ✓ Inter- and intra-assay reproducibility: <15% CV of calculated concentrations (in linear range of assay)
- ✓ Analyte cross-reactivity: <10%
- ✓ Benchmarking to ELISA

Our Targets list

HUMAN					MOUSE	
Adiponectin	IFN-α	IL-18	IL-13	MIP-1α	GM-CSF	LAP/TGFβ
CEA	IFNγ	IL-21	IL-17A	MIP-1β	Granzyme B	MCP-1
cMET	IgA	IL-4	IL-17F	SAA	IFNα	TNFα
EGF	IgE	IL-5	IP-10	Tau pT181	IFNγ	VEGF
Eotaxin	IgG4	IL-6	LAP/TGFβ	Tau Total	IL-1α	
EPO	IgM	IL-7	Leptin	TNFα	IL-2	
Flt-3 Ligand	IL-1α	IL-8	MCP-1	TNF-β	IL-4	
Fractalkine	IL-1β	IL-10	MCP-2	Troponin I	IL-5	
GM-CSF	IL-2	IL-12p40	MCP-3	VEGF	IL-6	
GRO-α	IL-3	IL-12p70	MDC		IL-17A	

Coming soon

HUMAN	MOUSE
IL-2R	IFNβ
IL-22	IL-22

www.thermofisher.com/ProQuantum

Clinical research on COVID-19

Discovering elevated cytokines in severe cases

Lancet. 2020 Feb 15;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5. Epub 2020 Jan 24.

Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.

Huang C[1], Wang Y[2], Li X[3], Ren L[4], Zhao J[5], Hu Y[6], Zhang L[1], Fan G[7], Xu J[8], Gu X[7], Cheng Z[9], Yu T[1], Xia J[1], Wei Y[1], Wu W[1], Xie X[1], Yin W[6], Li H[2], Liu M[10], Xiao Y[4], Gao H[11], Guo L[4], Xie J[5], Wang G[12], Jiang R[3], Gao Z[13], Jin Q[4], Wang J[14], Cao B[15].

Author information

Erratum in
Department of Error. [Lancet. 2020]

- Researchers observed that in patient with a severe response **IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A and TNF-α**

Lung injury from cytokine storm

Nat Rev Immunol. 2020 Apr 6. doi: 10.1038/s41577-020-0305-6. [Epub ahead of print]

In the eye of the COVID-19 cytokine storm.

Vaninov N[1].

Author information

1 Sinai Immunology Review Project, Icahn School of Medicine at Mount Sinai, New York, NY, USA. sinai.immunology@gmail.com.

- Cytokine storm shown to be a cause for the lung damage

Clinical trials using hMSCs to reduce cytokine levels

- As of 20th April 2020 there is current 45 cell therapy trials for Covid-19[1]
- Of which a vast majority uses hMSC[1]
- Recent references

Aging and Disease
www.aginganddisease.org
Volume 11, Number 2: 216-228, April 2020
http://dx.doi.org/10.14336/AD.2020.0228

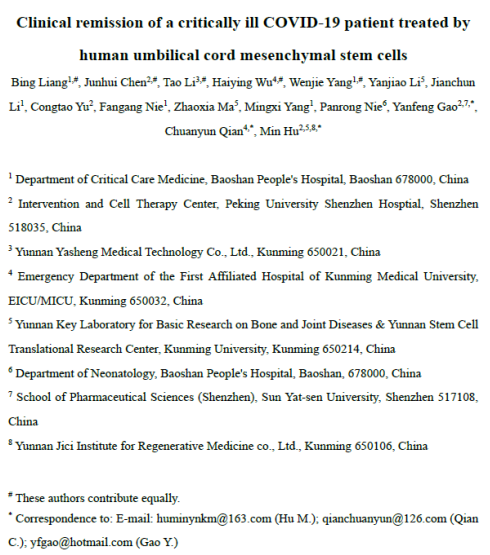
Original Article

Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia

Zikuan Leng[1,5,#], Rongjia Zhu[2,#], Wei Hou[3,#], Yingmei Feng[3,#], Yanlei Yang[4], Qin Han[2], Guangliang Shan[2], Fanyan Meng[1], Dongshu Du[1], Shihua Wang[2], Junfen Fan[2], Wenjing Wang[3], Luchan Deng[2], Hongbo Shi[3], Hongjun Li[3], Zhongjie Hu[3], Fengchun Zhang[4], Jinming Gao[4], Hongjian Liu[5,*], Xiaoxia Li[6], Yangyang Zhao[2], Kan Yin[6], Xijing He[7], Zhengchao Gao[7], Yibin Wang[7], Bo Yang[8], Ronghua Jin[2,*], Ilia Stambler[9,10,11], Lee Wei Lim[9,10,12], Huanxing Su[9,10,13], Alexey Moskalev[9,10,14], Antonio Cano[9,10,15], Sasanka Chakrabarti[16], Kyung-Jin Min[9,10,17], Georgina Ellison-Hughes[9,10,18], Calogero Caruso[9,10,19], Kunlin Jin[9,10,20*], Robert Chunhua Zhao[1,2,9,10*]

[1]School of Life Sciences, Shanghai University, Shanghai, China. [2]Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, School of Basic Medicine Peking Union Medical College, Beijing, China. [3]Beijing YouAn Hospital, Capital Medical University, Beijing, China. [4]Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [5]Department of Orthopaedics, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. [6]Institute of Stem Cell and Regeneration Medicine, School of Basic Medicine, Qingdao University, Shandong, China. [7]Department of Orthopaedics, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. [8]Department of Neurosurgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. [9]The Executive Committee on Anti-aging and Disease Prevention in the framework of Science and Technology, Pharmacology and Medicine Themes under an Interactive Atlas along the Silk Roads, UNESCO, Paris, France. [10]International Society on Aging and Disease, Fort Worth, Texas, USA. [11]The Geriatric Medical Center "Shmuel Harofe", Beer Yaakov, affiliated to Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel. [12]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China. [13]Institute of Chinese Medical Science, University of Macau, Taipa, Macau, China. [14]Institute of Biology, Komi Science Center of Russian Academy of Sciences, Syktyvkar, Russia. [15]Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Valencia, Spain. [16]Maharishi Markandeshwar Deemed University, Mullana-Ambala, India. [17]Department of Biological Sciences, Inha University, Incheon, South Korea. [18]Faculty of Life Sciences & Medicine, King's College London, London, UK. [19]Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy. [20]University of North Texas Health Science Center, Fort Worth, TX76107, USA.

[Received February 25, 2020; Revised February 28, 2020; Accepted February 28, 2020]



Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells

Bing Liang[1,#], Junhui Chen[2,#], Tao Li[3,#], Haiying Wu[4,#], Wenjie Yang[1,#], Yanjiao Li[5], Jianchun Li[1], Congtao Yu[2], Fangang Nie[1], Zhaoxia Ma[5], Mingxi Yang[1], Panrong Nie[6], Yanfeng Gao[2,7,*], Chuanyun Qian[4,*], Min Hu[2,5,8,*]

[1] Department of Critical Care Medicine, Baoshan People's Hospital, Baoshan 678000, China
[2] Intervention and Cell Therapy Center, Peking University Shenzhen Hosptial, Shenzhen 518035, China
[3] Yunnan Yasheng Medical Technology Co., Ltd., Kunming 650021, China
[4] Emergency Department of the First Affiliated Hospital of Kunming Medical University, EICU/MICU, Kunming 650032, China
[5] Yunnan Key Laboratory for Basic Research on Bone and Joint Diseases & Yunnan Stem Cell Translational Research Center, Kunming University, Kunming 650214, China
[6] Department of Neonatology, Baoshan People's Hospital, Baoshan, 678000, China
[7] School of Pharmaceutical Sciences (Shenzhen), Sun Yat-sen University, Shenzhen 517108, China
[8] Yunnan Jici Institute for Regenerative Medicine co., Ltd., Kunming 650106, China

[#] These authors contribute equally.
[*] Correspondence to: E-mail: huminynkm@163.com (Hu M.); qianchuanyun@126.com (Qian C.); yfgao@hotmail.com (Gao Y.)

1. https://celltrials.org/

How Thermo Fisher can partner you with MSC workflow for COVID-19

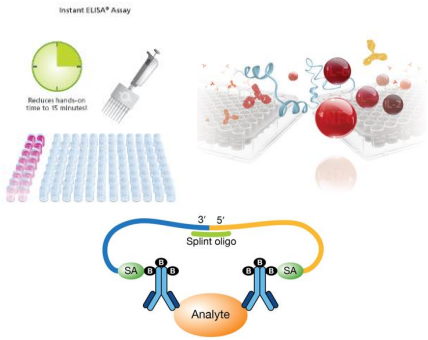
MSC Expansion

- Cell culture plastics
  - Nunc™ TripleFlask™ Treated Cell Culture Flasks
  - Cell Factory or bioreactor
- Reagents
  - StemPro™ MSC SFM XenoFree
  - CTS™ GlutaMAX™-I
  - CTS™ CELLstart™
  - CTS™ TrypLE™
  - CTS™TGFB1/FGF2 Recombinant Human Protein

Wash and fill

- Wash, media exchange, formulate, fill
  - Rotea™ counterflow centrifugation

Potency testing

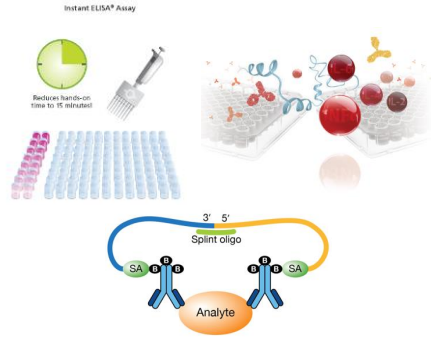


- Cytokine testing with
  - Instant ELISA
  - ProcartaPlex
  - ProQuantum

Purity and Safety testing

- Safety and purity testing
  - ™ MycoSEQ™ Mycoplasma Detection Assay ProQuantum
  - TaqMan Gene Expression Arrays
  - Attune NxT for cellular analysis
  - PIERCE LAL endotoxin kit

Patient severity classification



- Cytokine testing with
  - Instant ELISA
  - ProcartaPlex
  - ProQuantum

ThermoFisher SCIENTIFIC

Complementary RUO and CTS Products for MSC expansion

Seamless transition from basic research to clinical applications

- Our Research Use Only (RUO) portfolio supports cost-effective basic research
- Transition to Gibco™ Cell Therapy Systems (CTS™) products as you translate into clinical research

RUO	CTS
KnockOut DMEM/F-12	CTS KnockOut DMEM/F-12
KnockOut DMEM	CTS KnockOut DMEM
GlutaMAX Supplement	CTS GlutaMAX-I Supplement
TGF-β 1 Recombinant Human Protein	CTS TGF-β 1 Recombinant Human Protein
Synth-a-Freeze Medium	CTS Synth-a-Freeze Medium
TrypLE Select Enzyme	CTS TrypLE Select Enzyme
DPBS	CTS DPBS

Cell Processing System Specifically Developed for Cell Therapy Manufacturing

Gibco™ Rotea™ Counterflow centrifuge

Key benefits

- **Process flexibility**—user-programmable software enables you to create and optimize a broad range of protocols for cell separation, washing, and concentration
- **High cell recovery and viability**—gentle fluidized bed supports low shear processing, enabling >95% cell recovery with no loss in cell viability
- **Low output volumes**—proprietary technology that can deliver as little as 5 mL of concentrate
- **Appropriate for research through commercial manufacturing**—closed single-use kit; processing speed and compact size enable seamless commercial scale-up

Process Flexibility- A Multi-purpose System for Cell Therapy Manufacturing

Currently suitable for wash/concentration between cell therapy processing workflow steps

Cell therapy processing

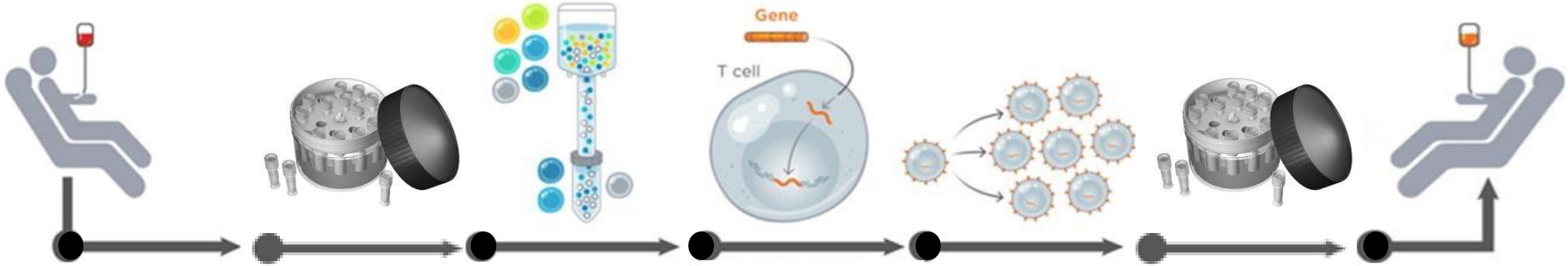
Thaw cells | Cell isolation and activation | Cell engineering | Cell expansion | Fill, finish, and cryopreservation | Lot release and characterization

Future applications can include isolation

Isolate PBMC from apheresis product

Isolate individual immune cell types (eg: T or NK cells)

Isolate engineered cells (eg: CAR-T, CAR-NK)



The Complete Gibco™ Rotea™ System

A complete, closed cell processing system

Instrument

- Compact
- Multipurpose
- Easily integrates into your process

Single-use kit

- Sterile, closed kit
- Flexible input and output ports

Software

- Intuitive, user-programmable interface
- Preset protocols available
- Supports GMP manufacturing

Highly flexible instrument and software with sterile, single-use kit that scales with you from research through commercial manufacturing

Takeaway message

START IT RIGHT AND EARLY

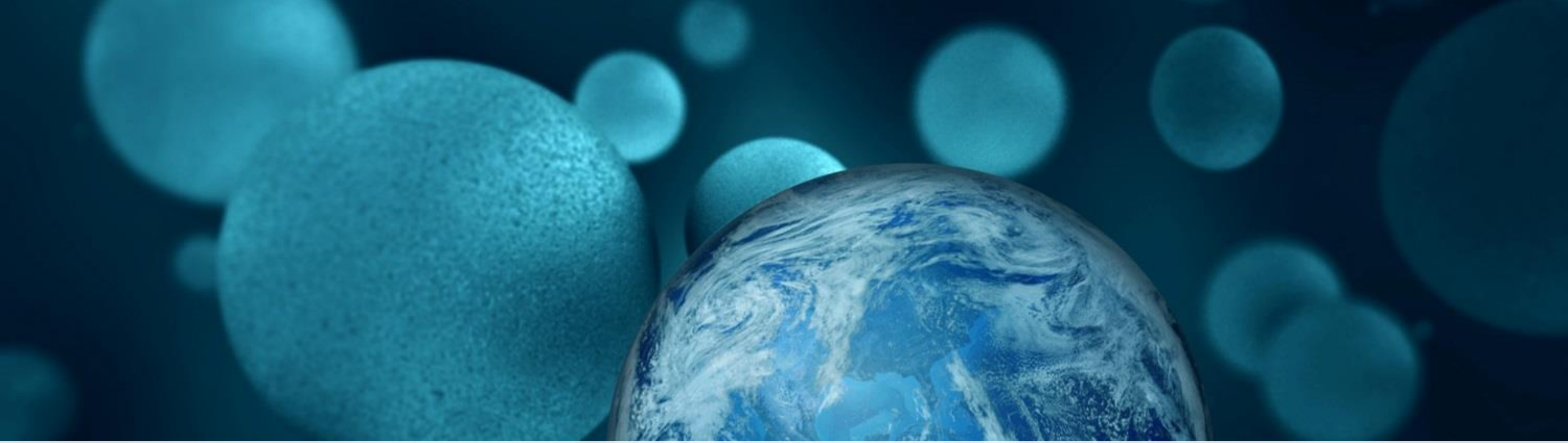
- Using the right starting material and reagents to prevent future issues
- Consider potency early, know what you want to test

CYTOKINE EXPRESSION IS CRITICAL FOR MULTIPLE ASPECTS

- The need to measure and establish cytokine signature for potency and safety

MAINTAIN CONSISTENCY WITH A CHEAPER, SHORTER AND FASTER WORKFLOW

- Shorter work flow, higher throughput



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Q&A