

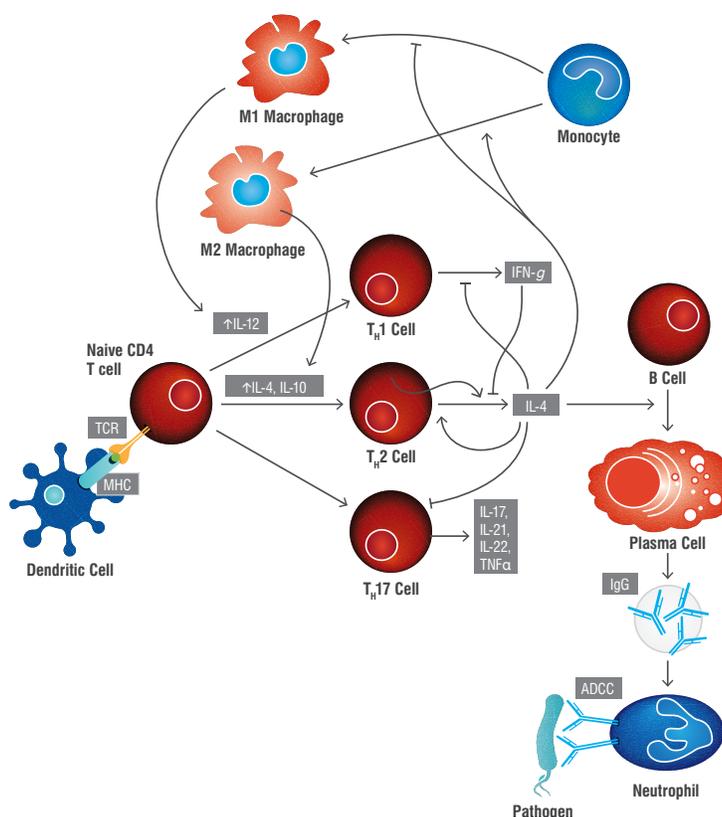
TruCulture: A Simple Whole Blood Collection and Culture System for Clinical Immunology Studies

Introduction

Rules-Based Medicine (RBM), a Q² Solutions Company, the world's leading multiplex biomarker testing laboratory, has developed a standardized platform for immune-phenotyping studies that are important for pharmacodynamic (PD) analysis, drug dosing and safety, and characterization of immune dysregulation. TruCulture® is a simple, *ex vivo* whole blood culture system that models *in vivo* immune responses. TruCulture is standardized to perform with a high level of reproducibility across all users and clinical sites.

The TruCulture system combines whole blood collection and whole blood culture. Unlike culturing of peripheral blood mononuclear cells (PBMC), TruCulture allows the culturing of whole blood, retaining all non-mononuclear cells and circulating soluble factors that can impact immune responses. The use of this closed system requires no extensive manipulation of immune cells, no need for specialized equipment or technical expertise, and can be performed at the site of collection.

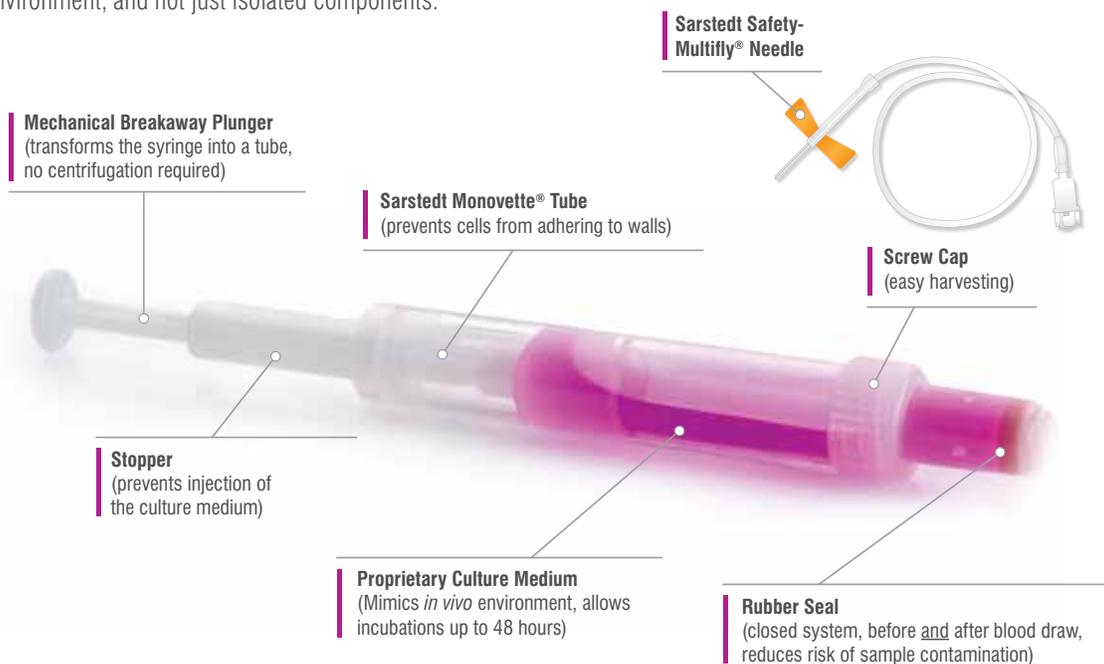
TruCulture responses can be analyzed using a variety of techniques and can be conveniently paired with the multiplexed immunoassay services at RBM. TruCulture is a complete solution for clinical immunology and immunology research.



Advantages of whole blood culture for clinical studies

TruCulture is a whole blood culturing system compared to the current industry standard of using isolated leukocyte populations. Culturing of whole blood mimics *in vivo* inflammatory responses by incorporating the complexity of the immune system with systemic factors (ie. hormones, microbiota products, drugs, etc.). Especially important for clinical research of biologics, whole blood cultures contain all cells that express Fc receptors, allowing analysis of any potential interactions between antibody-based drug targets and their innate receptors. Whole blood cultures also contains all circulating factors, including anything secreted from tissues at various sites and individual specific factors that could influence immune cell activation and function. For clinical dosing, safety, and PD studies, culturing of whole blood allows analysis of the entire *in vivo* circulating environment, and not just isolated components.

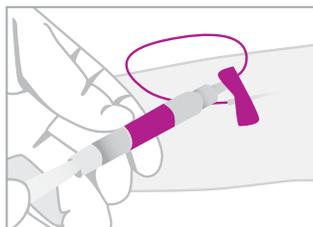
TruCulture is a whole blood culture system ideal for clinical PD investigations that can be tailored to the study. TruCulture medium can be supplemented with almost any soluble substance to target the immune pathway of interest. The custom substance can be a proprietary drug, known stimulator(s) and/or inhibitor(s) of immune pathways. Currently, TruCulture tubes are available with common stimulants (ie. LPS, anti-CD3/CD28, TLR agonists, etc.) that have been optimized for use in clinical studies. For new compound(s), a custom TruCulture product will undergo a detailed development process, which can be tailored to any study (please refer to white paper “TruCulture®: Custom Development Service for Clinical Pharmacodynamic (PD) Studies”).



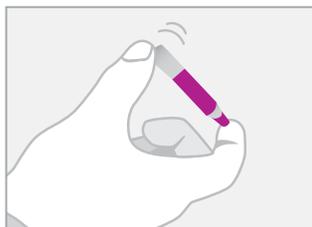
TruCulture – Designed for simplicity

TruCulture tubes are pre-loaded with proprietary culture medium and can be used by anyone trained to draw blood. After 1 mL of whole blood is collected directly into the tube, the syringe plunger is removed, transforming the syringe into a cell culture tube. Gentle inversion is all it takes to thoroughly mix the whole blood and the culture medium. The culture tube is incubated in a dry heat block at 37°C for up to 48 hours. **No CO₂ incubator required.**

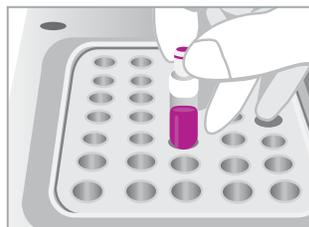
After the stationary incubation period, the cells will have settled to the bottom of the tube, the valve separator is inserted, separating the cells from the supernatant. **No centrifuge required.** Once the stick is removed, the entire tube, with the supernatant and the cells separated by the valve separator can be stored at -20°C and transported to RBM for downstream analysis.



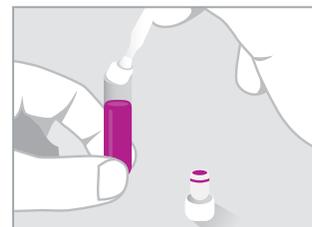
1. COLLECT



2. MIX



3. INCUBATE



4. SEPARATE

The TruCulture system is designed to work seamlessly with RBM's biomarker testing services, offering a simple solution for PD applications. As a CLIA certified immunoassay testing facility, RBM offers multiple immune panels based on different platforms. OptiMAP is a single cost-effective multiplex assay optimized for TruCulture and consists of 13 analytes chosen to give a comprehensive profile of the immune response with a variety of TruCulture immune stimulants. HumanMAP allows detection of analytes from multiple immune pathways simultaneously. In cases when analysis of low concentration analytes are needed, such as for antigen recall applications, the ultrasensitive Simoa™ platform is available. The combination of TruCulture with standardized and reproducible biomarker testing services enables clinical researchers to address challenging questions in the complex world of immune-related disease biology.

In addition to the biomarker assays offered by RBM, TruCulture cultured cells, once separated from the supernatant, can be collected for a variety of downstream analyses, including gene expression analysis by NanoString™, RNAseq, or RT-PCR and cell marker analysis by flow cytometry. Customers may consult with an RBM scientist for protocol development.

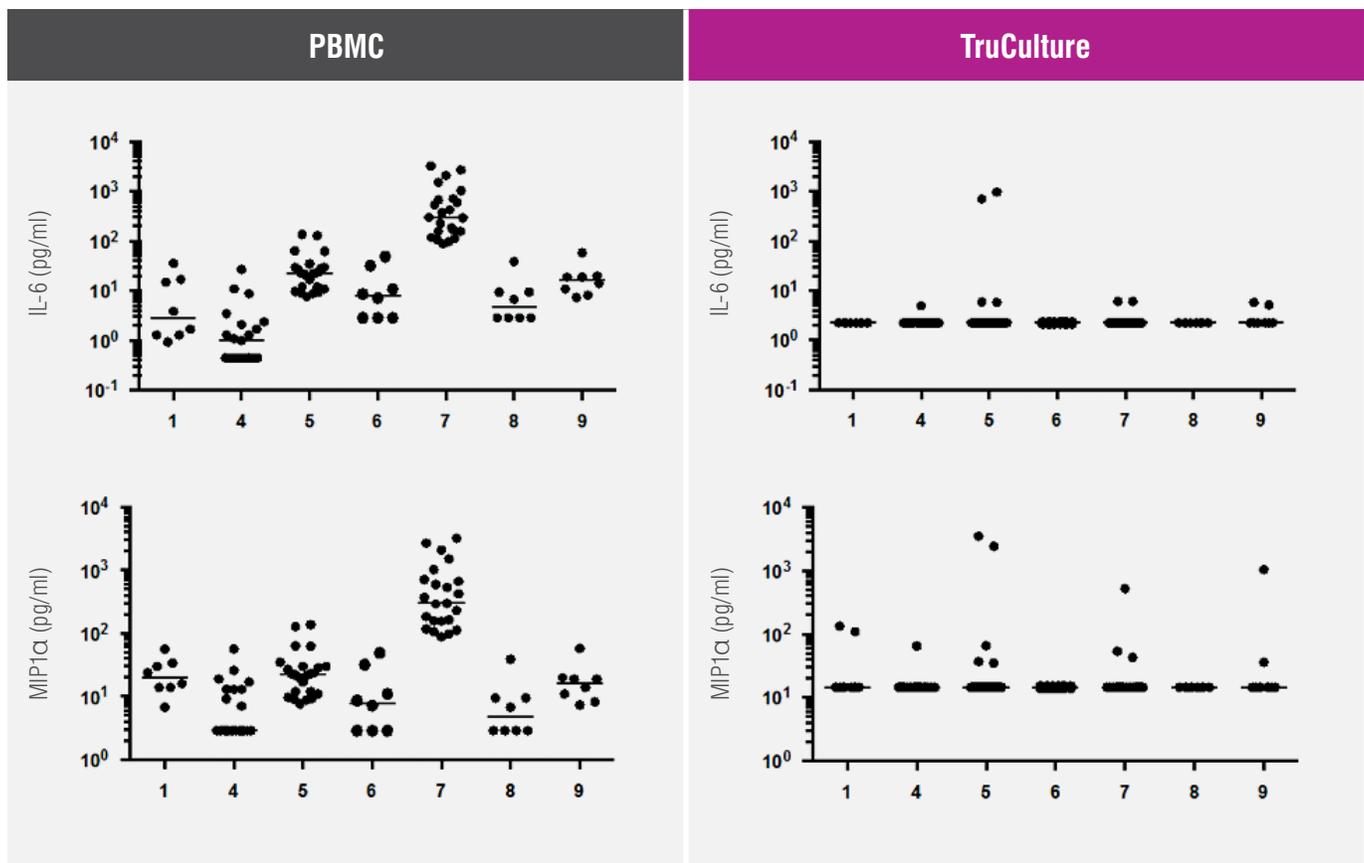
TruCulture system has been optimized for use in clinical trials

TruCulture tubes are developed and manufactured under rigorous quality standards, assuring consistent performance among users and study sites.

TruCulture reduces nonspecific immune activation

TruCulture tubes are loaded with a specially designed culture medium that allows efficient immune cell culturing in a closed system with just a dry heat block. The TruCulture tubes can sustain whole blood cells for up to 48 hours with no adverse occurrence that can activate inflammatory responses: 1) leukocyte cell death, 2) coagulation, and 3) lysis of RBCs. Additionally, TruCulture requires no extensive cell manipulation prior to culture and minimizes processing stressors that can activate innate inflammation. Compared to PBMC cultures, cells cultured in TruCulture tubes without any stimulants remain minimally activated.

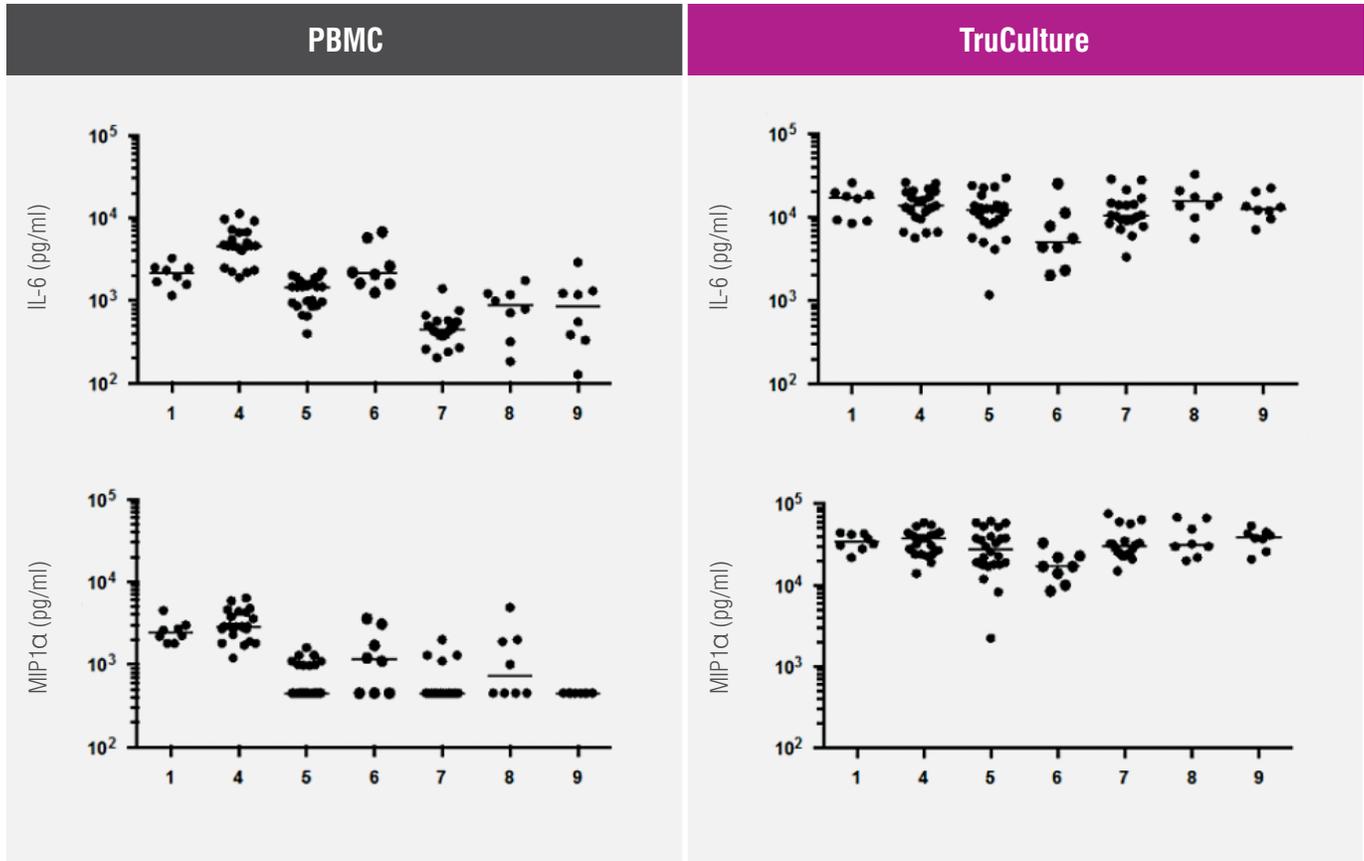
In 7 different testing centers, donor samples were split into the PBMC or TruCulture groups and cultured in medium (no stimulants) for 22 hours. Supernatants were analyzed by RBM HumanMAP technology. (Darragh Duffy *et al.* Clinical Immunology 2017)



TruCulture provides high reproducibility and stimulation across clinical sites

TruCulture also reduces variability by eliminating the need for sample processing prior to culturing. The extensive standardization and validation process for each TruCulture system ensure its performance will remain consistent between users and clinical sites. In a multi-center trial coordinated by the Institut Pasteur, TruCulture consistently demonstrated superior reproducibility and consistency of data compared to PBMC cultures. Additionally, whole blood cultured in TruCulture demonstrated higher stimulation of immune factors.

In 7 different testing centers, donor samples were split into the PBMC or TruCulture groups and stimulated with LPS for 22 hours. Supernatants were analyzed by RBM HumanMAP assay. For the 35 proteins measured, 29 showed variance between centers in the PBMC group while only 4 proteins were found to be variable between centers with TruCulture (ANOVA $q < 0.01$) (Darragh Duffy *et al.* Clinical Immunology 2017).



TruCulture – Utilization in clinical research

TruCulture is a key resource for immunology and immuno-oncology research and has been utilized in various clinical studies, ranging from 1) evaluating PD responses to immune therapeutics, 2) determining efficacy of novel compounds, and 3) identifying dysregulated immune pathways.

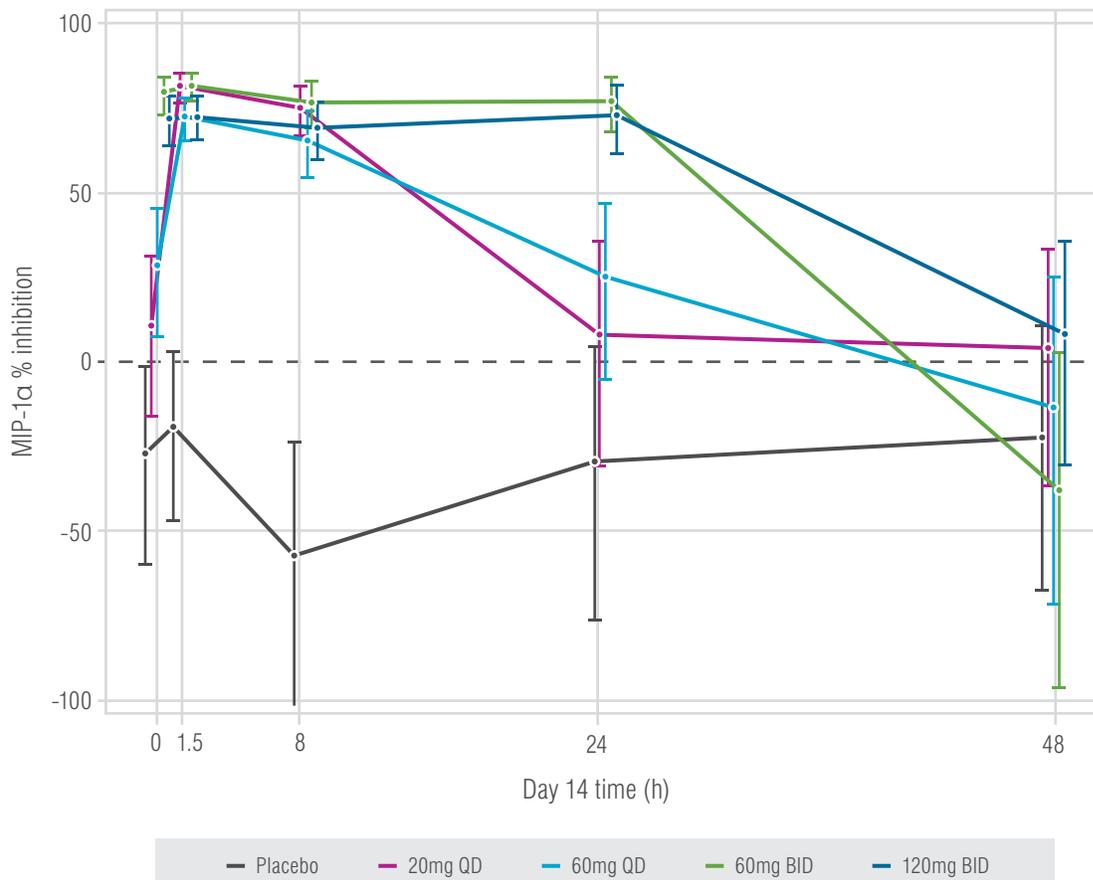
Example 1: Randomized clinical study of safety, pharmacokinetics, and pharmacodynamics of RIPK1 Inhibitor GSK2982772 in healthy volunteers

Kathleen Weisel et al. Pharmacology Research Perspectives 2017

Receptor-interacting protein kinase 1 (RIPK1) is involved in mediating necroptosis, also called programmed necrosis. This process of necroptosis is now believed to play critical roles in several inflammatory diseases, including rheumatoid arthritis and psoriasis. GSK2982772 is a RIPK1 inhibitor that prevents RIPK1-mediated cell death and cytokine production. This study is the first human study to determine PD of single and repeated doses of GSK2982772 in healthy adult males.

The PD study was examined using TruCulture tubes supplemented with a cocktail (TNF- α , zVAD, and Smac mimetic) to stimulate necroptosis. The cocktail includes tumor necrosis factor alpha

(TNF- α); z-VAD-fmk (zVAD), a pan caspase inhibitor; and the second mitochondria-derived activator of caspase (Smac) mimetic, which sensitizes caspase deficient cells for TNF- α induced cell death. Volunteers received 1) placebo, 2) 20mg once daily (QD), 3) 60mg once daily (QD), 4) 60mg twice daily (BID), or 5) 120mg twice daily (BID) for 14 days. At the end of the 14th day, whole blood was collected from each volunteer at 0, 1.5, 8, 24 and 48 hours post-dose into the TruCulture tubes and incubated for 6 hours. The supernatants were collected and analyzed for production of MIP-1 α . The figure below demonstrates that GSK2982772 inhibited RIPK1 dependent MIP-1 α production. The maximum inhibition was observed at 1.5 and 8 hours post treatment for all treatment groups.



TruCulture products

TruCulture tubes are available with or without stimulant(s) and can also be customized to suit any customer need. Standard tubes are usually in stock and are immediately available upon order submission.

Standard Tubes	Stimulant Target	Part Number
Null	N/A	782-001086
Lipopolysaccharide (LPS)	TLR-4	782-001087

Made-to-order tubes include products that contain stimulants that have gone through extensive development and previously ordered by customers. These are made on a per order basis.

Made to Order	Stimulant Target	Part Number
TStim	T cell	782-001416
Anti-CD3	T cell	782-001202
Anti-CD3 + Anti-CD28	T cell	782-001125
Zymosan	TLR2 Ligand	782-001259
Fibroblast-stimulating Lipopeptide (FSL-1)	TLR2 Ligand	782-001274
Lipoarabinomannan (<i>M.smegmatis</i>)	TLR2 Ligand	782-001280
Polyinosinic:polycytidylic acid (poly I:C)	TLR3 Ligand	782-001282
Gardiquimod (GDQ)	TLR7/8 Ligand	782-001269
Resiquimod R848 (R848)	TLR7/8 Ligand	782-001264
Lauroyl-g-D-glutamyl-meso-diaminopimelic acid (C12-iEDAP)	NOD Ligand	782-001273
Adenosine Triphosphate + LPS-EB	NLRP3 Inflammasome, TLR4	782-001272
Interferon beta (IFN- β)	Cytokine	782-001277
Tumor Necrosis Factor alpha (TNF- α)	Cytokine	782-001295
Interleukin-1 beta + tumor necrosis factor-alpha (IL-1 β + TNF- α)	Cytokine	782-001278

Conclusion

TruCulture is the only whole blood collection and culture system designed for use in clinical studies by modeling the human *in vivo* immunity in a simple, easy-to-use *ex vivo* system. TruCulture is easily implemented and used at multiple clinical study sites, delivering consistent and reproducible results. By pairing immunoassay analysis from RBM, TruCulture is a valued partner for immunology and immuno-oncology studies.

Contact us

Website: RBM.Q2LabSolutions.com/TruCulture

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