



truCulture[®]
INSTRUCTIONS FOR USE



TruCulture is For Research Use Only. Not Intended for Use in Diagnostic Procedures.

TruCulture is covered by the following patents:

US6410334B1, AU199954193A, EP1102988A2, AT308045T, DE59912716D1

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Intended Use

TruCulture® is a whole blood culture system incorporating proprietary media and a mechanical separation system. Blood cells are cultured and then separated from media for analysis of soluble or cellular components.

This product is for research use only. It is not intended for use in diagnostic procedures or patient management.

Background

The immune system consists of a complex, multi-layered signaling network that provides specific and sensitive responses to stimuli. Responses can vary greatly between individuals yet patterns for any given individual are remarkably consistent if standardization of sample collection and minimization of sample manipulation are employed. For accurate immunomonitoring data, it is essential that one conserve as close to *in vivo* conditions in an *in vitro* system.

Product Description

TruCulture is a simple, self-contained whole blood culture tube supplied with or without an immune system stimulant. After blood collection and incubation, a valve is manually inserted to separate the cells from the supernatant. The specimen can then be prepared for analysis according to the desired downstream applications, i.e. use the supernatant for protein biomarker analysis or use the cellular fraction for gene expression analysis. The TruCulture tubes have been developed and optimized for protein biomarker analysis using Myriad RBM's InflammationMAP® testing service that measures 46 protein biomarkers, chemokines, and acute phase reactants. Gene expression analysis results will vary depending on the methods used.

BLOOD COLLECTION AND HANDLING

Equipment Provided for Specimen Collection

1. TruCulture® syringe-tube with stimulated or NULL media
2. S-Monovette® (priming tube)
3. Safety-Multifly® 19G – 21G
4. Valve filter Seraplas V11

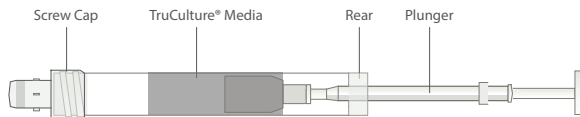
Equipment Not Provided

1. Dry Block heater capable of maintaining consistent 37°C temperature, external calibration device recommended.
2. Heat blocks with 12 MM to 13 MM diameter and a hole depth of 40-55 mm (e.g. VWR Catalog # 13259-130 or VLM Catalog # V.69153.61820).
3. Tube rack to freeze specimens upright. No Styrofoam racks should be used.
4. If sample analysis will not be completed by Myriad RBM, the following supplies may be necessary depending on your application:
 - a. Protein biomarker analysis
 - i. Transfer pipettes and tubes for supernatant
 - ii. Vortex
 - iii. Centrifuge capable of spinning down cells (400 – 500 g, brake turned off)
 - iv. Reagents and equipment required for analysis
 - b. Gene Expression
 - i. Transfer pipettes
 - ii. Transfer tubes
 - iii. Reagents and equipment required for analysis
5. Eye protection, gloves and other personal protective

equipment as necessary for prevention from exposure to blood borne pathogens using standard precautions.

Prevention of Backflow

TruCulture® tubes contain serum-free cell culture media with or without immune stimulants. It is important to avoid possible backflow from the tube to prevent the possibility of the media entering the blood circulation. To protect against backflow, pull the syringe plunger back until it clicks and locks safely during venipuncture. Do not push the syringe plunger in for any reason. The Safety-Multifly® available for use with the TruCulture system provides additional protection from backflow.



Recommended Draw Order

1. Use the S-Monovette® priming tube provided to fill the Multifly® tubing system completely with blood.
2. TruCulture tubes must be collected immediately thereafter to avoid activation of platelets.
3. If stimulated and NULL TruCulture tubes will both be collected, the stimulated tubes must be drawn first.
4. Any other tubes included in the study can be drawn from the same needle after filling the TruCulture tubes.

Warnings and Precautions

1. Practice Universal Precautions. Use gloves, gowns, eye protection, other personal protective equipment, and engineering controls to protect from blood splatter, blood leakage, and potential exposure to bloodborne pathogens.

2. Handle all biologic samples and blood collection “sharps” (lancets, needles, luer adapters, and blood collection sets) according to standard guidelines and the policies and procedures of your facility. Obtain appropriate medical attention in the event any exposure to biologic samples (for example, through a puncture injury), since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases.
3. Discard all blood collection “sharps” in biohazard containers approved for their disposal.
4. Never open the screw cap of the TruCulture® tubes before the end of the culture period.
5. TruCulture tubes are intended for single use. Do not try to re-use.
6. After using TruCulture tubes, be sure to handle, store, and ship the tubes using appropriate measures and labels that clearly indicate the potential infectious hazards of human blood.

Storage

TruCulture tubes should be stored at -20°C until ready for use.

Do not store TruCulture tubes in -70°C or -80°C freezers before use.

All other TruCulture System components should be stored at room temperature.

Specimen Collection and Culture Instructions

1. Follow universal precautions during blood collection to minimize exposure hazard.
2. Thaw the required number of TruCulture tubes for 1 hour at room temperature, in a non-styrofoam or insulating rack. Never thaw the tubes at >37°C. After thawing, TruCulture tubes cannot be refrozen and should be

discarded if blood is not drawn within 24 hours.

3. Label the tubes as appropriate. Do not place labels over the original TruCulture® label.
4. Prior to drawing blood, press the plunger into the TruCulture tube until it stops.
5. Using the Multifly® needle system provided, connect its adaptor to the front end of an empty S-Monovette® syringe (priming tube) and lock it by turning clockwise.
6. Puncture the vein, ensure the cannula position is safe and the blood flows easily. Release any tourniquets used to access vein. Draw just enough blood to fill the tubing system of the multifly needle set completely.
7. Remove the S-Monovette (priming tube) and replace with the first TruCulture tube.

Note: *Do NOT, for any reason, depress the plunger after the TruCulture tube has been attached to the inserted Multifly set.*

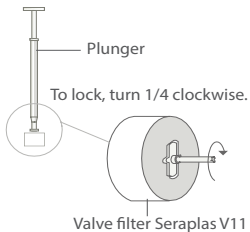
8. Fill the TruCulture tube slowly with blood by pulling the syringe plunger gradually until it snaps into its final position with a gentle click.
9. Wait for 5 seconds until the blood volume shows no further increase.
10. Disconnect the TruCulture tube from the Multifly adaptor and gently mix the tube contents by inverting 3 times end over end, being careful to avoid foaming.
11. Break away the plunger close to the rear of the TruCulture tube.
12. Remove any blood remaining in the tube cap by gently tapping the bottom of the TruCulture tube on the bench top.
13. Place in 37°C block thermostat with the tube-cap end point up.

- Repeat steps 8 through 13 to fill additional TruCulture® tubes, if required.
- Remove cannula when the desired number of tubes have been filled.
- Incubate all TruCulture tubes at 37°C in the block thermostat (or equivalent) for a study-defined period of time, preferably not to exceed 48 hours. The study-defined time should be strictly adhered to for all specimens of the same cohort. Any deviations should be noted, and it is recommended that the exact start and stop time of the cultures are recorded for each tube.
- If the study-defined incubation time is less than 24 hours, centrifugation may be necessary to sediment layers. If clear layers are not visible after incubation, spin the tubes at 400 – 500 g for 10 min using a centrifuge with the brake off.

Specimen Separation and Preparation Instructions

Note: *It is important to keep the TruCulture tubes in an upright position during this procedure.*

- Within 10 minutes prior to ending the incubation in step 16, assemble the Valve filter Seraplas V11 by inserting the plunger into the slot of the separator and lock them with a clockwise turn.
- Carefully remove the TruCulture tubes from the incubator. Avoid shaking.
- Remove the screw cap from each tube and slowly insert the assembled Seraplas valve until it is about 5 mm (1/4 inch) above the cell sediment level.



21. Disconnect and remove the plunger from the Valve filter Seraplas V11 with a counter-clockwise turn. The valve will stay in the TruCulture® tube.

Note: *If specimens are intended for gene expression analysis, remove supernatant and collect cell layer in the appropriate lysis buffer prior to freezing in order to preserve nucleic acid integrity. Please contact Myriad RBM at info@MyriadRBM.com for our recommended procedure.*

22. Close the TruCulture tubes with the screw caps (hand-tight).
23. Freeze the TruCulture tubes at -20°C immediately, in an upright position.
24. If tubes are being shipped to an external lab for protein biomarker analysis, ship them on dry ice, in an upright position. Do not use Styrofoam racks.
25. If tubes are being shipped to Myriad RBM for Multi-Analyte Profiling (MAP®) analysis, ship frozen tubes upright on dry ice following the instructions outlined in the Human Sample Submission form located at www.myriadrbm.com/order/how-to-order.

In-house or Alternative Lab Protein Biomarker Analysis

26. If tubes will be analyzed for protein biomarker analysis in house or at a facility other than Myriad RBM, thaw the tubes in an upright position prior to beginning the analysis.
27. Pour off the supernatant into a separate tube, removing as much of it as possible without disturbing the valve.
28. Vortex the tube with the supernatant.
29. Spin the supernatant at 400 g for 10 min to remove any particulates.
30. Begin applicable protein biomarker analysis protocol.

Ordering TruCulture®:

TruCulture tubes are provided with or without a stimulant. The table below describes the validated stimulants available, and the cells that are targeted by those stimulants. Custom stimulant TruCulture tubes are also available. Please inquire.

NULL Tubes (part # 782-001086) and NegCo Tubes (Part #782-001291) do not contain any stimulants and may be utilized for experimental control.

If you are interested in adding a custom compound or placing an order for TruCulture®, please contact your sales representative, or e-mail info@myriadrbm.com.

PART #	STIMULUS	DESCRIPTION
782-001086	Null	Pure (proprietary) TruCulture media without stimulants
782-001087	Lipopolysaccharide (LPS)	Bacterial endotoxin (E.coli, O55:B5) that elicits a strong innate immune response
782-001089	LPS + Staphylococcal Enterotoxin B (SEB)	This combination elicits a coordinated activation of T-cells (SEB) and cells of the innate immune system (LPS)
782-001124	Staphylococcal Enterotoxin (SEB)	Bacterial superantigen, stimulating polyclonal T-cell responses via the T-cell receptor (Th1 bias)
782-001125	anti-CD3 + anti-CD28	Two antibodies triggering T-cell activation via the signaling unit of the T-cell receptor complex (CD3) + co-activation (intensifying T-cell responses, adding activities of Th2 and Treg) via CD28
782-001126	SE-B + CD28	Bacterial superantigen, stimulating polyclonal T-cell responses via the T-cell receptor (Th1 bias) + co-activation (intensifying T-cell responses, also those of Th2) via CD28
782-001202	anti-CD3	T-cell activation via the signaling unit of the T-cell receptor complex (CD3)
782-001224	Intron A (IFN-alpha)	Type I interferon, modulator of for example T lymphocyte responses
782-001225	Intron A + LPS-EB high	IFN-alpha modulates the response of cells of the innate immune system to LPS
782-001259	Zymosan	β -glucan particles (fractions of yeast cell walls); stimulates phagocytes

PART #	STIMULUS	DESCRIPTION
782-001261	LPS-EB high	Bacterial endotoxin (E.coli, O111:B4) that elicits a strong innate immune response
782-001264	Resiquimod R848	Synthetic agonist of TLR7 and TLR8 (both responding to single-stranded RNA)
782-001269	Gardiquimod	Synthetic agonist of TLR7 (responding to single-stranded RNA, for example)
782-001272	Adenosine Triphosphate (ATP) + Lipopolysaccharide (LPS-EB) in high concentration	ATP modulates via purinergic receptors (such as P2X7) LPS-induced activation of cells of the innate part of the immune system
782-001273	Lauroyl- γ -D-glutamyl-meso-diaminopimelic acid (C12-iE-DAP)	Dipeptide representing bacterial peptidoglycan, activator of NOD1 (intracellular pattern recognition receptor)
782-001274	Fibroblast-stimulating Lipopeptide (FSL-1)	Synthetic analogue of microbial lipoprotein; agonist of TLR2/TLR6
782-001275	HKEB	Heat killed preparation of the gram negative bacterium, E. Coli O111:B4. Stimulates Toll-Like Receptor 2 (TLR2)
782-001276	HKLR	Heat killed Lactobacillus rhamnosus. Stimulates TLR2
782-001277	Interferon beta (IFN-beta)	Type I interferon, modulator of for example T lymphocyte responses
782-001278	Interleukin-1beta (IL-1beta) + tumor necrosis factor-alpha (TNF-alpha)	2 synergistically acting pro-inflammatory cytokines (weak to modest immune cell activation)
782-001279	Intron A + anti-CD3 + anti-CD28	IFN-alpha modulates the T-cell response to anti-CD3 + anti-CD28
782-001280	Lipoarabinomannan from M. smegmatis (LAM-MS)	Lipoglycan in mycobacterial cell walls that activates macrophages through TLR-2.
782-001281	Class A CpG oligonucleotide (ODN 2216) + LPS-EB high	ODN is a synthetic oligonucleotide including CpG motifs that are common in bacterial DNA. ODN stimulates the immune system through TLR9.
782-001282	Polyinosinic:polycytidylic acid (Poly I:C)	Analogue of double-stranded RNA, mimics the presence of viral infection. Activator of TLR3.
782-001291	NegCo	TruCulture media without stimulants, specially formulated for premium and custom tubes
782-001295	TNF-alpha	Pro-inflammatory cytokine; weak activator of mediator synthesis when used alone

To order standard or custom stimulants for TruCulture® tubes, please contact Myriad RBM at +1-512-835-8026 or info@myriadrbm.com.

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