



truculture[®]
INSTRUCTIONS FOR USE

RULES  ***BASED MEDICINE***
a Q²Solutions Company

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 preserve, as much as possible, *in vivo* conditions in any *in vitro* analysis system.

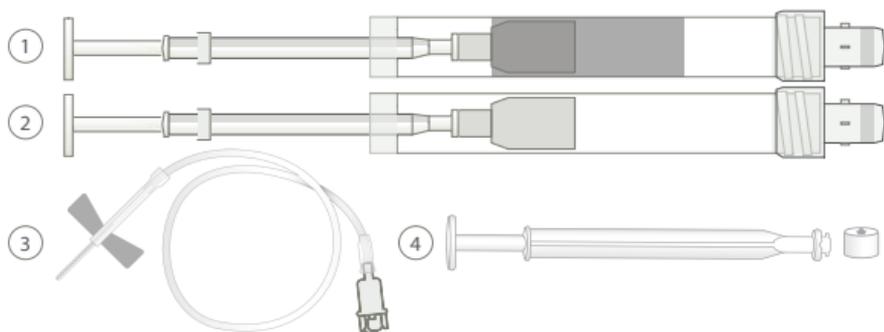
Product Description

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 is then prepared for analysis according to the desired downstream applications, i.e. supernatant for protein biomarker analysis (RBM protein biomarker testing services), the cellular fraction for gene expression, flow cytometry and other cell based analysis.

BLOOD COLLECTION AND HANDLING

Equipment Provided for Specimen Collection

1. TruCulture® syringe-tube
2. S-Monovette® (Priming Tube)
3. Safety-Multifly® 19G – 21G (Butterfly Needle)
4. Valve filter Seraplas V11 (Valve and Stick)



Equipment Not Provided

1. Dry Block heater (Heat Block) 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.

Warnings and Precautions

1. Practice Universal Precautions. Use personal protective equipment.
2. Handle and discard all biologic samples and blood collection sharps according to standard guidelines and the policies and procedures of your facility.

3. Prevention of Backflow. TruCulture tubes contain serum-free cell culture media with or without immune stimulants. The creation of a vacuum by pulling the syringe plunger back until it clicks and locks immediately before venipuncture limits the risk of backflow. **Once the vacuum has been established, do not push the syringe plunger in for any reason;** doing so may introduce substances into the donor that could lead to an adverse immune system reaction. The Safety-Multifly available for use with the TruCulture system provides additional protection from backflow.
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 re-use.
6. Handle, store, and ship the TruCulture samples according to standard guidelines and the policies and procedures of your facility.

Storage Prior to Use

1. TruCulture tubes
 - a. Store at -20°C Do not store tubes in -70°C or -80°C
2. TruCulture System components
 - a. Store at room temperature

Recommended Draw Order

1. Use the S-Monovette Priming Tube provided to fill the Safety-Multifly tubing system completely with blood.
2. TruCulture
 - a. Stimulated Tubes should be drawn first.
 - a. Then Null (unstimulated) TruCulture tubes.

3. Any other non TruCulture tubes included in the study can be drawn from the same needle after filling the TruCulture tubes.

Specimen Collection and Culture Instructions

1. Turn on the Heat Block and allow to equilibrate to 37°C.
2. Thaw the required number of TruCulture tubes for 1 hour at room temperature. Never thaw the tubes at >37°C. After thawing, TruCulture tubes should not be refrozen.
3. Label the tubes according to study specifications. Do not place labels over the original TruCulture label. Ensure the TruCulture stimulant type matches on both the TruCulture tube and the label you are placing on the tube.
4. Prepare the S-Monovette (Priming Tube) and all TruCulture tubes by pulling the syringe plunger until it clicks into its final locked position, creating a vacuum.
5. Using the Safety-Multifly needle system provided, puncture the vein and ensure the cannula is in the proper position.
6. Connect the prepared S-Monovette (Priming Tube) to the Safety-Multifly adapter. Draw just enough blood to fill the Safety-Multifly tubing and remove the S-Monovette (Priming Tube) immediately.
7. Connect the first prepared TruCulture tube to the Safety-Multifly adapter. Wait until the blood volume shows no further increase.
8. Disconnect the blood filled TruCulture tube from the Multifly adapter and gently mix the tube contents by inverting 3 times end over end.
9. Repeat steps 7 and 8 to fill additional TruCulture tubes, if required.
10. Remove the Safety-Multifly system when the desired number of tubes have been filled.

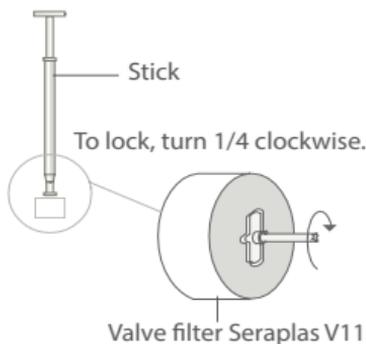
11. Break away the syringe plunger on each TruCulture tube.
12. Remove any blood remaining in the tube cap by gently tapping the bottom of the TruCulture tube on the bench top.
13. Place in 37°C Heat Block with the tube cap end pointing up. It is recommended to place the TruCulture tubes into the Heat Block within 15 minutes of blood collection.
14. Incubate all TruCulture tubes at 37°C in the Heat Block for the study defined period of time. It is recommended that incubation time does not exceed 48 hours.

Note: *If the incubation time is less than 12 hours, centrifugation (400 rcf for 10 minutes with brake off) may be necessary.*

Specimen Separation and Preparation Instructions

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

1. Within 10 minutes of completing the incubation, assemble the Valve filter Seraplas V11 by inserting the Stick into the slot of the Valve and lock with a clockwise turn.
2. Remove each TruCulture tube from the Heat Block, being careful to keep them upright, and do not disrupt the cell layers.
3. Remove the screw cap from each TruCulture tube and slowly insert an assembled Seraplas Valve until it is about 5 mm (1/4 inch) above the cell layer.
4. Disconnect the Stick from the Valve with a counterclockwise turn and remove the Stick from the Valve. The Valve will remain in the TruCulture tube. Close the TruCulture tube with its screw cap.



5. Repeat Specimen Separation and Preparation Instructions steps 2-4 for each TruCulture tube.
6. Freeze the TruCulture tubes at -20°C or below immediately, in an upright position.
7. If tubes are being shipped to RBM for analysis, ship frozen tubes on dry ice following the instructions outlined in the Human Sample Submission Guidelines located at www.rbm.q2labsolutions.com/order/how-to-order.

HOW TO ORDER

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.

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.

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
782-001125	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
782-001202	anti-CD3	T-cell activation via the signaling unit of the T-cell receptor complex (CD3)
782-001259	Zymosan	β -glucan particles (fractions of yeast cell walls); stimulates phagocytes
782-001261	LPS-EB	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)	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

PART #	STIMULUS	DESCRIPTION
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)
782-001281	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.
782-001411	SARS-CoV-2 Spike Protein	SARS-CoV-2 Spike Protein (2019-nCoV) S1 + S2 ECD.
782-001416	TStim	T cell stim stimulant + anti-CD28

To order standard or custom stimulants for TruCulture tubes, please contact RBM at (+01) 512 835 8026 or RBM_ClientServices@Q2LabSolutions.com.

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