LeukoComplete™ products

- ▶ LeukoComplete™ Plate and RT Kit
- ▶ LeukoComplete™ Gene Detection Kit
- ▶ LeukoComplete™ Antigen Coated Plate

For Research Use Only

Cellular immunity assay based on mRNA detection (EAGL assay)

Canon Medical Diagnostics Corporation

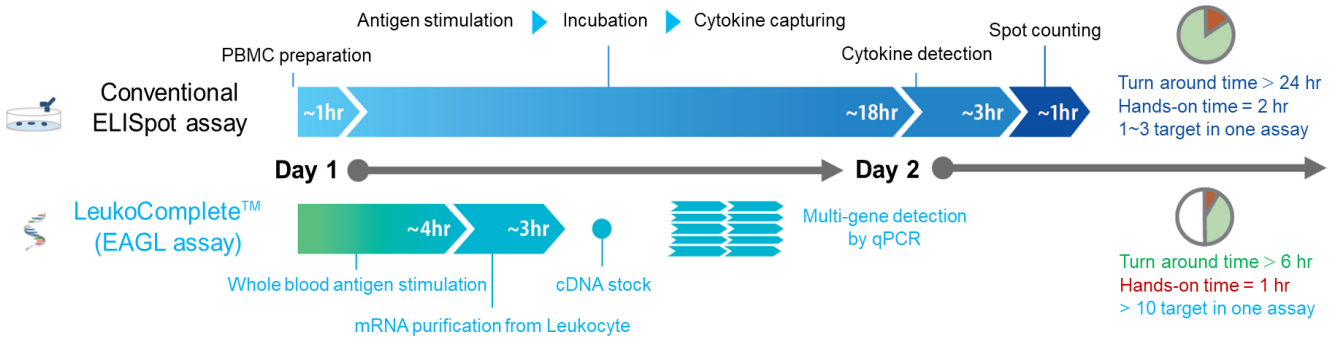
Revolutionizing Cell-Mediated Immune Response Analysis

The LeukoComplete™ Kit is an all-in-one solution for evaluating cell-mediated immune responses using cutting-edge RT-PCR technology. Designed for maximum efficiency and versatility, the LeukoComplete™ Kit offers significant advantages over traditional protein-based assays like ELISpot (Enzyme-Linked ImmunoSpot) and ICS (IntraCellular Staining), providing researchers with a faster, more sensitive, and more flexible approach to immune response analysis.

Why Choose LeukoComplete™?

▶ A Faster, Smarter Approach

Unlike traditional methods that require long incubation times, large sample volumes and labor-intensive PBMC isolation, the LeukoComplete™ Kit enables same-day analysis by detecting mRNA expression levels from whole blood samples. This faster turnaround time, combined with reduced sample requirements, makes it ideal for high-demand research environments.



▶ Applications and Flexibility

The LeukoComplete™ Kit is ideal for a broad range of applications, from antigen screening in **Vaccine development** to cancer and autoimmune disease research. Its proprietary **Leukocyte Isolation Plate[1]** and **mRNA Capture Plate[1]** ensure high performance with clinical samples, supporting advanced methods such as the **Ex Vivo Activation of Gene in Leukocyte (EAGL assay[2]).** Elevate your research with the LeukoComplete™ Kit—bringing precision, speed, and reliability to cell-mediated immune response analysis[3].

Academia

- **Want to screen a large number of antigen and adjuvant candidates**
- **Want to evaluate both aspects of protein and gene expression**
- **Want to evaluate responses that can only be seen in genes**

Pharma CRO

- **Considering the introduction of cellular immunoassays**
- **plan to evaluate a large number of samples for clinical/non-clinical studies, etc.**
- **Want to store evaluation samples and perform archival measurements**

Why Choose LeukoComplete™?

- **Whole Blood Compatibility:**
  Simplify your workflow with direct compatibility for whole blood samples, eliminating complex preparation steps.
- **Detection of Multiple Genes:**
  Assess a wide range of mRNA targets, including cytokines, to gain a comprehensive view of immune activity.
- **High-Throughput Analysis:**
  Optimize efficiency with the ability to process large sample volumes simultaneously.
- **High Sensitivity:**
  Amplify and detect even low-abundance mRNA with unparalleled accuracy using RT-qPCR and digital PCR.
- **Small Sample Volume Requirement:**
  Achieve reliable results with minimal sample input, preserving precious materials.
- **Normalization of Results:**
  Ensure consistent and reliable data with built-in normalization to reference genes.
- **cDNA Storage for Future Testing:**
  Archive synthesized cDNA for reanalysis, expanding experimental flexibility and downstream applications.
- **Independent of Antibody Availability:**
  Avoid dependency on antibodies by leveraging mRNA quantification for target detection.
- **Comprehensive All-in-One Kit:**
  Streamline every step, from cell isolation to gene detection, with our complete kit solution.

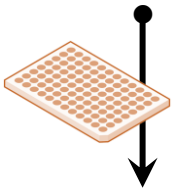
▶ Specification

Intended use	- Analysis of cell-mediated immune response - Detection of cytokine genes
Sample type	- Whole blood (Human, Mouse) - PBMC (Human, Mouse) - Spleen (Mouse) All samples can be either fresh or frozen.
Final product	30 µL cDNA
Target gene*	Reference gene (Human, Mouse) ・ACTB, GAPDH, B2M Target gene (Human) ・IFNG, IL2, TNFSF2、IL6, IL10, GM-CSF, CXCL10, IL4, IL5 Target gene (Mouse) ・IFNG, IL10, IL17
Instrument	Real-time qPCR Digital PCR

*Additional genes have been tested in house. Please contact us for more details.

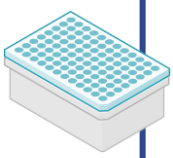
Protocol

Described below is a workflow with LeukoComplete™ products comprising 2 parts: cDNA preparation with **LeukoComplete™ Plate and RT Kit** (Part A) and RT-qPCR with **LeukoComplete™ Gene Detection Kit** (Part B).



Antigen stimulation of sample

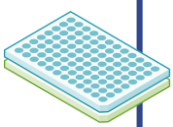
- **LeukoComplete™ Antigen Coated Plate** as an option for the study of SARS-CoV-2
- Stimulation with heparinized blood sample should be conducted within 24 hr from blood draw.



Part A

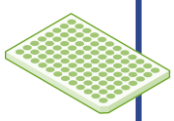
➢ Isolation of immune cells

Add PT buffer to Filter Plate → Add **Sample** to Filter Plate **(100 µL/well)** → Stand still (10 min) → Place mRNA Capture Plate on Filter Plate



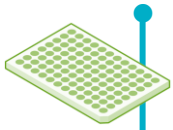
➢ mRNA extraction & purification

Add Lysis Buffer to Filter Plate → Stand still (30 min) → Stand still (60 min) → Wash mRNA Capture Plate with Wash Buffer A → Wash mRNA Capture Plate with Wash Buffer B



➢ Synthesis of cDNA

Add RT buffer to mRNA Capture Plate → Stand still (30 min) → **cDNA synthesis completed** → Store cDNA



Part B

➢ Detection of target gene

cDNA template → Mix cDNA template and PCR reagents → **PCR**

Turn Around Time
Part A: 180 min
Part B: 60 min/gene

● Data analysis

■ Validated target genes and their stimulation conditions

Target gene expression level of human sample when stimulated with various positive controls

Gene \ Antigen	PMA/IM	PHA	LPS	CEF	Anti-IgE
IFNG	+++	+++	+++	++	-
IL2	-	+	N.D.	+	-
TNFSF2	-	+++	-	+/-	-
IL4	-	+/-	N.D.	+/-	+++
IL5	-	+/-	N.D.	N.D.	N.D.
IL6	-	+++	+++	+/-	-
IL10	-	++	+	+	-
IL13	-	+++	+++	+/-	++
CSF2	+++	+++	+++	++	-
CXCL9	+++	+++	+++	++	-
CXCL10	N.D.	+++	+++	+	-

Rank	Ratio of reactive donors ($log_2FC > 2$)
+++	> 90%
++	60-90%
+	30-60%
+/-	0- 30%
N.D.	0%
—	Not evaluated

Abbreviation: PMA; Phorbol 12-myristate 13-acetate, IM; Ionomycin, PHA; Phytohemagglutinin, CEF; CEF control peptide pool, Anti-IgE; Anti-human IgE antibody for basophil activation test

Please refer to the product website for more information.

Analytical performance

■ High efficiency of reference gene extraction from multiple sample types

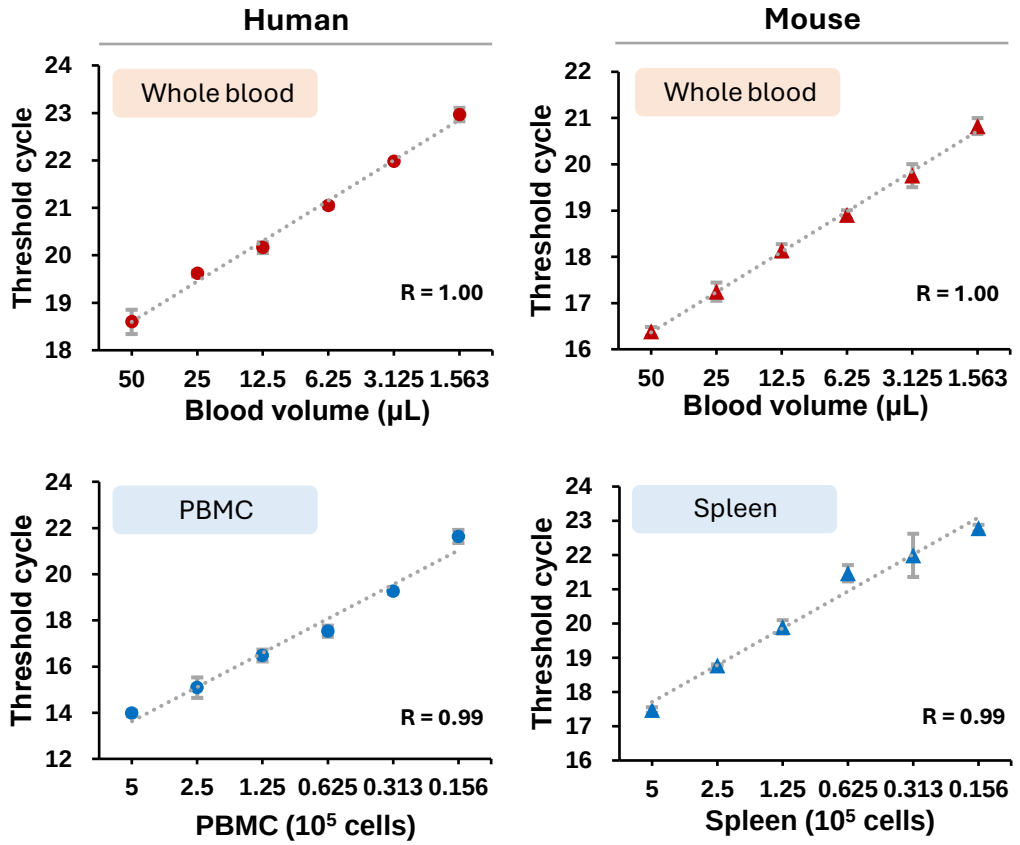


Figure 1. House keeping gene expression level from different sample types of various concentrations. Human whole blood (upper left), mouse whole blood upper right), human PBMC (lower left) and mouse spleen (lower right).

■ Short stimulation time for detection of change in target gene expression level

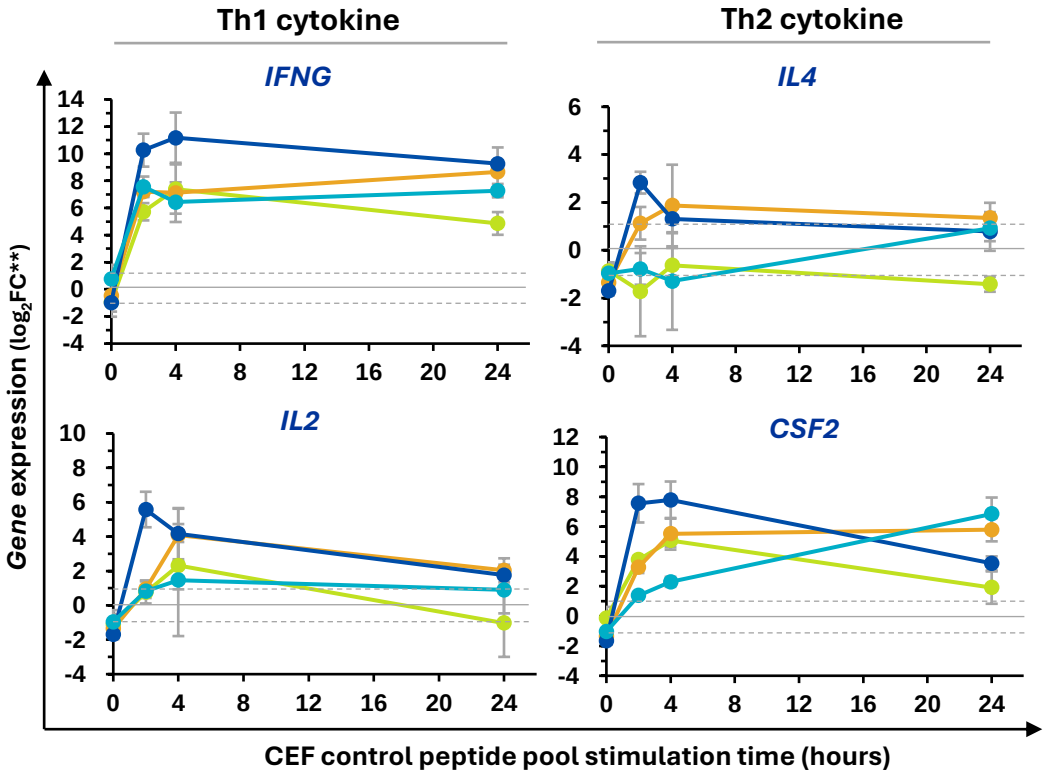


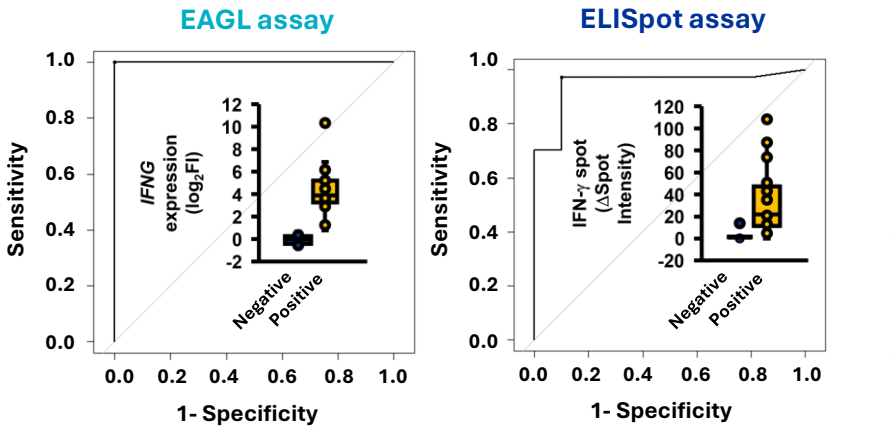
Figure 2. Change in target gene expression level at various stimulation time. 100 µL fresh human whole blood was stimulated with CEF control peptide* or PBS for 0, 2, 4, 24 hours at 37℃. Then the target genes: IFNG, IL2 (Th1 cytokines) or IL-4, CSF2 (Th2 cytokines) was detected from each sample. (n = 4) . The relative expression level of target genes against reference gene was calculated according to the equation 1.

* Peptide pool derived from CMV, EBV and Influenza virus.

** $\log_2 FC$ = – ((Ct value for target gene [stimulation+] – Ct value for reference gene[stimulation+])
– (Ct value for target gene [stimulation –]– Ct value for reference gene[stimulation –]))

Clinical performance

➢ Immune response with or without vaccination; A comparison with ELISpot [2]



Assay	EAGL	ELISpot
Total Positive donors	37	37
Total Negative donors	6	10
AUC	1.000	0.949
Cut off	0.720	3.818
Sensitivity	1.000	0.900
Specificity	1.000	0.973

Figure 3. Immune response against SARS-CoV-2 Spike-glycoprotein (SpG) overlap peptide from vaccinated patients with no known history of infection (n = 37) and unvaccinated patients (n = 10). Whole blood samples from the patients were stimulated with SpG overlap peptide for 4 hours at 37℃. Then EAGL assay was conducted. For ELISpot assay, PBMC from the patients were prepared and incubated with SpG overlap peptide for 72 hours at 37℃. The following assays were conducted according to the manufacturer's instruction.

Applications

➢ Screening of antigen [2]

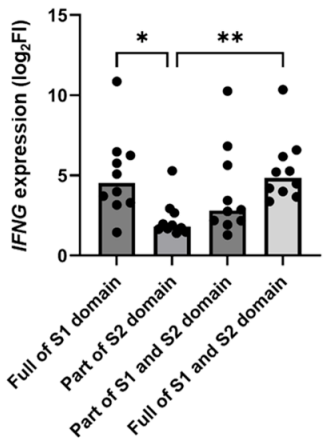


Figure 4. Relative expression level of INFG gene against ACTB from patients who were vaccinated with mRNA vaccine (n = 10). Whole blood samples from the patients were stimulated with SpG, which was used for the vaccine development. Overlap peptide of SpG (15 mer, 11 overlap) was used as antigen for the stimulation.

➢ Instead/comparison of other assays

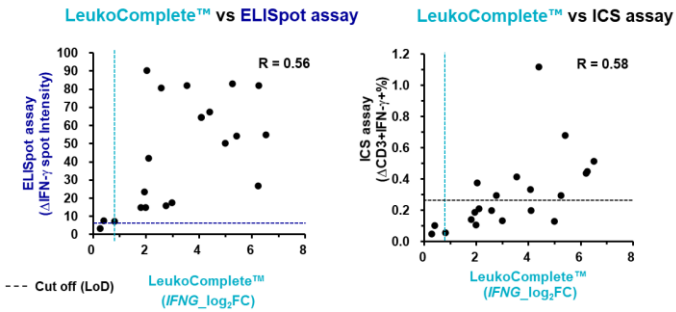


Fig. 5 Fresh blood or PBMCs from 21 COVID-19 vaccinees were used to evaluate cellular immune responses to overlapping peptides of the SARS-CoV-2 spike protein: IFN-γ gene (IFNG) for LeukoComplete™, IFN-g for ELISpot method, The ICS assay was performed on CD3-positive IFN-g-secreting cells.

➢ Relationship among breakthrough infection, clinical symptoms and cellular immune activity

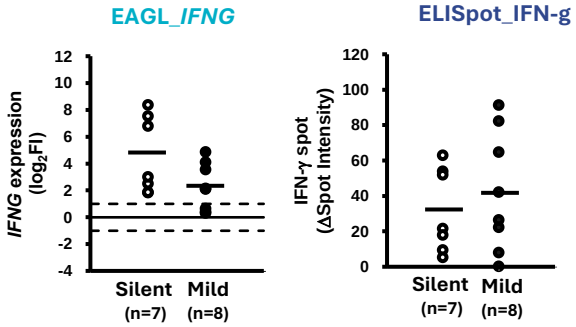


Figure 6. Immune response from vaccinated patients who experienced breakthrough infection or who did not experience it.

[3]

➢ Transcriptome analysis for optimal target gene selection from archived cDNA

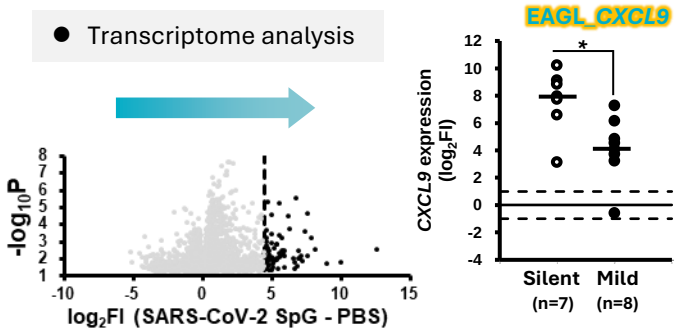


Figure 7. Transcriptome analysis revealed CXCL9 as best target gene to distinguish patient with breakthrough infection without clinical symptoms or patient with breakthrough infection with clinical symptoms.

[3]

Kit Component

■ LeukoComplete™ Plate and RT Kit

Component	Volume	Storage Temp.
PT Buffer	1 × 15 mL	2–30°C
Leukocyte Isolation Plate (Filter Plate)	1	2–30°C
Deep Well Plate	1	2–30°C
Proteinase K	1 × 30 µL	2–30°C
TCEP	1 × 300 µL	2–30°C
Lysis Buffer	1 × 6 mL	2–30°C
mRNA Capture Plate	1	2–30°C
Wash Buffer A	1 × 30 mL	2–30°C
Wash Buffer B	1 × 50 mL	2–30°C
Aluminum Seal	1	2–30°C
M-MLV Reverse Transcriptase	1 × 40 µL	≦ −20°C
RNase Inhibitor	1 × 10 µL	≦ −20°C
RT Buffer	2 × 1.5 mL	≦ −20°C

■ LeukoComplete™ Gene Detection Kit

Component	Volume	Storage Temp.
Primer Mix*	1 × 100 µL	≦ −20°C
PCR Enzyme Mix	1 × 500 µL	≦ −20°C

Target gene

Gene	Human	Mouse
IFNG	11150-ht-001	11150-mt-001
IL2	11150-ht-002	-
TNFSF2	11150-ht-003	-
IL4	11150-ht-004	-
IL5	11150-ht-005	-
IL6	11150-ht-006	-
IL10	11150-ht-007	11150-mt-002
IL13	11150-ht-008	-
IL17	-	11150-mt-003
CXCL9	11150-ht-009	-
CXCL10	11150-ht-010	-
CSF2	11150-ht-011	

Control gene

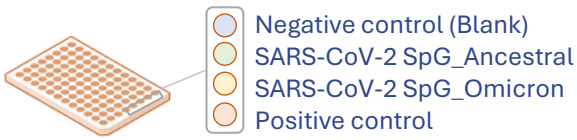
Gene	Human	Mouse
ACTB	11150-hc-001	11150-mc-001
GAPDH	11150-hc-002	11150-mc-002
B2M	11150-hc-003	11150-mc-003

* Independent Cat. # is assigned to each gene.
Please select target gene and control gene of interest.

■ LeukoComplete™ Antigen Coated Plate

Component	Volume	Storage Temp.
Antigen Coated Plate for SARS-CoV-2**	1 (24 test)	2–30°C

** Overlap peptide pool for spike protein from SARS-CoV-2 (15 a.a. , 11 overlap) Each test requires 4 wells as described in the right figure.



Information

● **Equipment needed, but not included**
Centrifuge swing rotor for 96-well plate, Incubator, Real-time PCR instrument

● **How to purchase**
This kit can be purchased from Cosmo Bio USA.

● **Reference**

[1] Mitsuhashi, M., et al., Clin Chem, 2006. 52(4): p.634-42.
[2] Saito, T., et al., Biochem Biophys Res Commun, 2023. 694: p.1493-98.
[3] Saito. T., et al., under Submission

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