

ADAMII™-CD3/CD4/CD8 Kit

T-Cell Counting System

REF CD348K-025

IVD For *in vitro* diagnostic use only

Intended use

ADAMII™-CD3/CD4/CD8 Kit is intended for *in vitro* diagnostic use on ADAMII image based fluorescence cell counter. It contains three-color direct immunofluorescence reagent to identify and determine the absolute counts of human T lymphocytes (CD3+), helper/inducer (CD3+/CD4+) T lymphocytes, and suppressor/cytotoxic (CD3+CD8+) T lymphocytes in RBC-lysed whole blood. The test is not intended for use in point-of-care settings.

Summary and explanation of test

T-cells (CD3+ cells) have been shown to play a role in the pathogenesis of GVHD,¹ and T-cell depletion from the donor graft is the most effective prophylaxis of GVHD. However, it has been associated with a higher rate of graft failure, relapse and prolonged T-cell immunodeficiency, with increased fatal opportunistic infections.² Donor lymphocyte infusions (DLI), an effective treatment for relapsed chronic myeloid leukemia (CML) after allogeneic stem cell transplantation, are not successful when Leukemia resistance and secondary graft-versus-host disease (GVHD) occur via T-cell mediated immune response.³ The CD3+CD4+ and CD3+ CD8+ are Helper/inducer and Suppressor/cytotoxic lymphocytes subset of T lymphocytes (CD3+). CD3+CD4+ and CD3+CD8+ counts are used to monitor the immune status of human immunodeficiency⁴⁻⁶ and autoimmune diseases.^{7,8} ADAMII T-cell Counting System provides accurate absolute number of CD3+/CD3+CD4+/CD3+CD8+ cells with rapid turnaround and minimal hands-on time for the operator. The high degree of precision of the system is due in part to the simplifying sample preparation steps, elimination of washing and reconstituting steps which may cause cell loss and human error. In addition, results are reported using test specific Software, limiting interpretation by user.

Principle of the assay

ADAMII™ T-cell assay starts with adding an appropriate volume of a sample to a single test tube and mixing with the reagent solution containing fluorescence-labeled antibodies. When the sample is mixed in the test tube, fluorescence-labeled antibodies bind specifically to the CD3+, CD4+ and CD8+ cell surface. Once the staining step is completed, RBC sLysis Buffer is added for the lysis of red blood cells. The lysed sample is loaded into a disposable plastic slide and the slide is placed on the precision stage of ADAMII™ instrument, an image-based fluorescence cell counter based on the LED excitation and CCD detection technologies. Focus can be adjusted by the move focus button and starting image captures of the stained cells will trigger automatic processes, generating (1) CD3+ cells/ μL , (2) CD3+/CD4+ cells/ μL , (3) CD3+/CD8+ cells/ μL , (4) T H/S ratio which are displayed on the result screen.

Material provided

The ADAMII-CD3/CD4/CD8 Kit(CD348K-025) 1 Box consists of:

Q'ty	Contents	Catalogue number
1	ADAMII™ CD3/CD4/CD8 Reagent (25 tests)	A348R-001
1	ADAMII™ 10X RBC sLysis Buffer (4 mL)	A348L-001
1	ADAMII™ Calibration Beads (25 tests)	A2CB-001
25	ADAMII™ Assay Slide	A2AS-25
1	ADAMII™ CD348-Kit Package Insert	-

Materials required but not provided

- Reagent-grade (deionized) water
- EDTA blood collection tubes or equivalent
- Microcentrifuge tube
- Calibrated micropipettor with tips capable of delivering 5 μ L
- Calibrated micropipettor with tips capable of delivering 20 μ L
- Calibrated micropipettor with tips capable of delivering 100 μ L
- 1X PBS (Dulbecco's modified, pH 7.2 \pm 0.2) with 0.5% bovine serum albumin (BSA), if sample dilution is necessary.
- Tubes for sample staining and RBC lysis buffer dilution
- Micropipette and tips
- Timer / Vortex mixer
- Personal protective equipment and biohazard waste disposal containers
- Control kit (manufacturer's recommendations)

Warnings and Precautions

- For *in vitro* diagnostic use only
- Do not use the reagent or Assay Slide if you observe any change in appearance.
- Do not decontaminate ammonium chloride lysed samples with bleach.
- To achieve an accurate result, it is critical to add a precise volume (20 μ L) of specimen to an empty tube and mix with a precise volume (5 μ L) of ADAMI[™] CD3/CD4/CD8 Reagent solution containing antibodies.
**Use reverse pipetting or a positive displacement pipette to aliquot samples.*
**See the pipette manufacturer's instructions for more information.*
- Ammonium chloride lysing solution is harmful if swallowed (R22) and irritating to the eyes (R36). Wear suitable protective clothing, eyewear, and gloves. Dispose of in accordance with federal, state, and local regulations.
- All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.
- When using lysis buffer, use after dilution.
- After using Calibration Beads, Reagent, 10x RBC Lysis buffer, close the cap tightly.

Storage and Stability

All closed/open materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for 12 months from the date of manufacture. The expiration date is clearly indicated on the product box, pouch, tube and bottle.

Material	Catalogue number
Refrigerator temperature storage (2~8 °C)	
- ADAMII™ CD3/CD4/CD8 Reagent	A348R-001
- ADAMII™ Calibration Beads	A2CB-001
- ADAMII™ 10X RBC Lysis Buffer	A348L-001
Ambient temperature storage (2~25 °C)	
- ADAMII™ Assay Slide	A2AS-25

Specimen collection and handling

- Anticoagulated blood stored at room temperature(20°C - 25°C) must be stained within 48 hours of draw.
- Laboratory should validate their pre-analytical sample storage conditions.
- Specimens should be analyzed within 3 hours after staining.
- Do not use previously fixed and stored specimens for more than 48 hours.
- Do not use clotted, or clumped specimens.
- It is recommended that hemolyzed samples not be tested.
- ADAMII CD3/CD4/CD8 Reagent has been validated with just EDTA anticoagulants. It has not been validated for use with heparin or acid citrated dextrose(ACD) liquid anticoagulants in determining absolute counts with ADAMII™-CD3/CD4/CD8 Kit.
- Before sample staining, adjust the number of total white blood cells (WBC) of sample up to 150,000 cells/μL by diluting sample with 1XPBS. Dilution factor may be entered for automatic calculation at the sample type selection menu.

Procedures

Calibration

The Calibration Beads (7.3 μm) for the ADAMII™ instrument is included in the kit are fluorescent particles with specific size and fluorescence dyes, which enable user to check if the ADAMII™ instrument is in good condition in terms of optic alignment and software algorithm to count cells by processing images from multiple different channels.

The calibration beads must be run on the ADAMIITM Instrument at least once each day of use. Refer to the ADAMIITM Instrument User Manual for complete instructions.

Quality control

In accordance with the Good Laboratory Practices and laboratory regulations, running two levels of cellular control material (procedural control) is recommended. These should be processed like patient samples to monitor the ongoing performance of the entire analytic process. Control should be run at least once each day that patient testing is performed. Commercial controls providing established values for absolute counts of CD3+ and CD3+/CD4+ and CD3+/CD8+ cells from a variety of manufacturers are available.

Specimen processing

1. Preparation

- (1) Label an empty tube and an ADAMIITM Assay Slide for the specimen identification.
- (2) Transfer 20 μ L of well-mixed specimen to the empty tube, add 5 μ L of ADAMIITM CD3/CD4/CD8 Reagent, cap the tube and mix well with vortex or finger flick.
- (3) Incubate for 10 minutes in the dark at room temperature (68~77°F, 20°C~25°C).
- (4) Dilute 10X RBC sLysis buffer to a new empty tube to prepare for 1X RBC sLysis buffer.

⚠ Note: Each day, prepare enough 1X sLysis buffer for use. To make, dilute 1 part of 10X sLysis buffer with 9 parts of distilled water. Store and use at room temperature (68~77°F, 20°C~25°C).

- (5) Add 100 μ L of 1X RBC sLysis buffer to the tube and vortex for 2-3 seconds.
- (6) Incubate for 3 minutes in the dark at room temperature (68~77°F, 20~25 °C).

2. Assay procedure

- (1) Load 25 μ L of the stained sample onto the ADAMIITM Assay Slide.

⚠ Caution: Vortex the cells thoroughly, at low speed, to mix cells well and to reduce the number of aggregations before loading stained cells on an assay slide.

- (2) Wait 3 minutes for sample to settle.
- (3) Insert the sample loaded ADAMIITM Assay Slide into the ADAMIITM Instrument and press 'Insert' button.
- (4) Go to the "Measure" menu and click the move focus button to adjust the focus in bright and fluorescence filed.

- (5) Select the sample type to capture, input the file name to be saved, and press “Run”.
- (6) the counting results will be displayed, and the slide is automatically ejected.

Procedural notes

- For an accurate result, it is critical to make use of the reverse pipetting technique to add an exact amount of sample to the tube and ADAMIITM Assay Slide.
- Insufficient mixing of samples will result in inaccurate results.
- Avoid bubbles at all times of sample loading onto the slide for an accurate result.
- Erroneous results will occur if tubes are not immediately placed and kept in the dark and cold condition, or if samples are exposed to the RBC lysis solution more than three hour prior to the counting.
- Fixed and stored specimens should be avoided. Avoid using hemolyzed, clotted, or clumped specimens.

Calculation or results

ADAMIITM Instrument performs all image capturing and analysis operations automatically once the sample loaded Assay Slide has been manually inserted into the ADAMIITM Instrument and “Run sample” is clicked. When it is done, four count results will be generated and displayed (1) CD3+ cells/ μ L, (2) CD3+/CD4+ cells/ μ L, (3) CD3+/CD8+ cells/ μ L, (4) T H/S ratio are also calculated.

Performance characteristics

Method comparison – Accuracy

CD3+ cells/ μ L, CD3+/CD4+ cells/ μ L, CD3+/CD8+ cells/ μ L counts were determined with the ADAMI-CD3/CD4/CD8 Kit on whole blood compared to the BD TritestTM CD4/CD8/CD3 using the FACSCalibur.

Figure 1 Total T lymphocytes CD3+ cells/ μ L

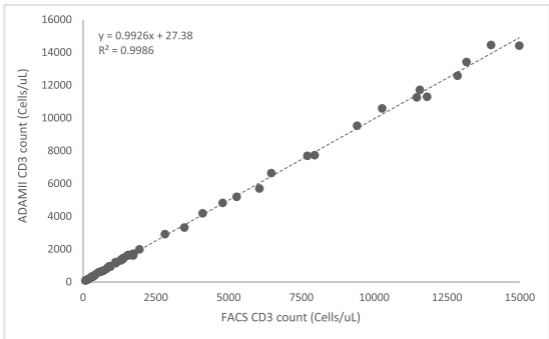


Figure 2 Helper/inducer T lymphocytes CD3+/CD4+ cells/ μ L

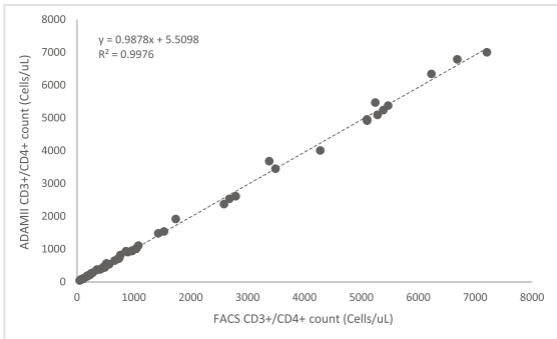


Figure 3 Suppressor/cytotoxic T lymphocytes CD3+/CD8+ cells/ μ L

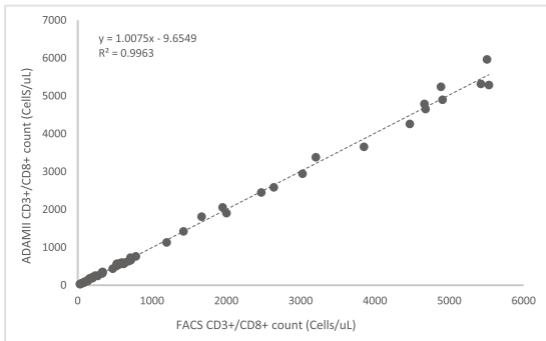


Table 1 Regression analysis

Subset	n	slope	intercept	R ²	Range
Total T lymphocytes (cells/ μ L)	50	0.99	27.38	1.00	78.56 -14980.76
Helper/inducer T lymphocytes (cells/ μ L)	50	0.99	5.51	1.00	44.52 -7204.80
Suppressor/cytotoxic T lymphocytes (cells/ μ L)	50	1.01	9.65	1.00	28.15 -5532.00

Precision(Within-run)

A single site testing was conducted using 6 levels of whole blood. One lot of reagent and one operator/instrument per tested 3 replicates per sample over 6 runs in 24 hours (sample stability limit).

Target concentrations of total T lymphocytes CD3+ cells/ μ L:

- 130 cells/ μ L
- 700 cells/ μ L
- 1,500 cells/ μ L
- 2,000 cells/ μ L
- 4,500 cells/ μ L
- 9,500 cells/ μ L

Target concentrations of helper/inducer T lymphocytes CD3+/CD4+ cells/ μ L:

- 50 cells/ μ L
- 400 cells/ μ L
- 1,000 cells/ μ L
- 2,300 cells/ μ L
- 4,600 cells/ μ L
- 6,400 cells/ μ L

Target concentrations of suppressor/cytotoxic T lymphocytes CD3+/CD8+ cells/ μ L:

- 60 cells/ μ L
- 300 cells/ μ L
- 860 cells/ μ L
- 1,700 cells/ μ L
- 2,300 cells/ μ L
- 4,000 cells/ μ L

Table 2 Precision Results

Subset	Target concentration	Mean	SD	CV(%)
Total T lymphocytes (cells/ μ L)	130	134.52	7.56	5.62
	700	697.02	24.29	3.48
	1,500	1463.18	59.78	4.09
	2,000	2015.78	96.66	4.80
	4,500	4419.10	146.71	3.32
	9,000	9492.66	154.29	1.63
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Subset	Target concentration	Mean	SD	CV(%)
Heppler/inducer T lymphocytes (cells/ μ L)	50	56.62	4.13	7.29
	400	407.23	25.65	6.30
	1,000	1006.79	50.09	4.98
	2,300	2290.21	74.77	3.26
	4,600	4604.27	119.45	2.59
	6,400	6365.87	131.87	2.07

Subset	Target concentration	Mean	SD	CV(%)
Suppressor/cytotoxic T lymphocytes (cells/ μ L)	60	57.66	5.24	9.08
	300	307.72	21.20	6.89
	860	866.72	49.29	5.69
	1700	1721.47	67.43	3.92
	2300	2374.40	82.51	3.47
	4000	4086.21	135.30	3.31

Linearity

To Linearity was assessed at the NanoEntek Research laboratory using eight target concentrations of CD3+ and CD4+ and CD8+ cells/ μ L:

- CD3+ : 120, 630, 1260, 2400, 3500, 5800, 8100, 11500
- CD3+/CD4+ : 70, 300, 600, 1160, 1700, 2800, 3900, 5500
- CD3+/CD8+ : 55, 250, 500, 940, 1400, 2200, 3100, 4500

Three replicates were stained and tested within one hour.

The ADAMII™-CD3/CD4/CD8 Kit demonstrated linearity across the claimed ranges:

- CD3+ : >120 to $<1.15 \times 10^4$
- CD3+/CD4+ : >70 to $<5.5 \times 10^3$
- CD3+/CD8+ : >55 to $<4.5 \times 10^3$

Limitations


















ADAMII™-CD3/CD4/CD8 Kit is designed for use on the ADAMII™ Instrument. Refer to the ADAMII™ Instrument User Manual for more information.

Do not use the ADAMII™-CD3/CD4/CD8 Kit or slides beyond the expiration date.

References

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3. Radujkovic A, Guglielmi C, Bergantini S, Iacobelli S, van Biezen A, Milojkovic D, Gratwohl A, Schattenberg AV, Verdonck LF, Niederwieser DW, de Witte T, Kröger N, Olavarria E. Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant.* 2015 21(7):1230-1236.
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Glossary of Symbols

	Caution, warning, Consult accompanying documents
	Catalogue number/Reference number
	Consult Instructions for Use An electronic instructions for use (eIFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.
	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
	Authorized representative in the European Community
	CE marking
	<i>In vitro</i> diagnostic medical device
	UK Conformity Assessment
	Temperature limitation
	Contains sufficient for <n> tests
	Do not reuse
	Do not use if package is damaged
	For prescription use only CAUTION: Federal (U.S.) law restricts this device to sale by or on order of a physician.
	US Corporation
	Instructs the end-user that the contents of the package requires dilution



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Revised on 2023.04