

## Overview

### Useful For

A second-level test after lymphocyte proliferation to mitogens (specifically phytohemagglutinin) has been assessed. The anti-CD3 proliferation panel is not a first-level test for assessing lymphocyte (T-cell) function

Evaluating patients suspected of having impairment in cellular immunity

Evaluation of T-cell function in patients with primary immunodeficiencies, either cellular (DiGeorge syndrome, T-negative severe combined immunodeficiency [SCID], etc) or combined T- and B-cell immunodeficiencies (T- and B-negative SCID, Wiskott Aldrich syndrome, ataxia telangiectasia, common variable immunodeficiency, among others) where T-cell function may be impaired

Evaluation of T-cell function in patients with secondary immunodeficiency, either disease related or iatrogenic

Evaluation of recovery of T-cell function and competence following bone marrow transplantation or hematopoietic stem cell transplantation

Evaluation of T-cell function in patients receiving immunosuppressive or immunomodulatory therapy

Evaluation of T-cell function in the context of identifying neutralizing antibodies in patients receiving therapeutic anti-CD3 antibody immunosuppression for solid organ transplantation or autoimmune diseases, such as type 1 diabetes

### Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
ADSTM	Additional Flow Stimulant	No, (Bill Only)	No

### Testing Algorithm

Testing with 2 stimulants will always be performed. When adequate specimen is available, a third stimulant will be performed and billed separately. If isolated patient's peripheral blood mononuclear cells (PBMCs) have a low WBC, selected dilutions or stimulants may not be used at the discretion of the laboratory to ensure the most reliable results.

### Method Name

Flow Cytometry

### NY State Available

Yes

## Specimen

### Specimen Type

WB Sodium Heparin

### Shipping Instructions

**Specimens are required to be received in the laboratory weekdays and by 4 p.m. on Friday.** Draw and

package specimen as close to shipping time as possible. Ship specimen overnight in an Ambient Shipping Box-Critical Specimens Only (T668) following the instructions in the box.

It is recommended that specimens arrive within 24 hours of draw.

Samples arriving on the weekend and observed holidays may be canceled.

### Necessary Information

**Date and time of draw and ordering physician's name and phone number are required.**

### Specimen Required

For serial monitoring, we recommend that specimen draws be performed at the same time of day.

**Supplies:** Ambient Shipping Box-Critical Specimens Only (T668)

**Container/Tube:** Green top (sodium heparin)

#### Specimen Volume:

<3 months: 1 mL

3 months-5 years: 3 mL

6-18 years: 5 mL

>18 years: 20 mL

**Collection Instructions:** Send specimen in original tube. **Do not aliquot.**

Blood Volume Recommendations Based on Absolute Lymphocyte Count (ALC)			
ALC	Blood Volume for Minimum aCD28 Only	Blood Volume for Minimum of aCD3, aCD28, and IL-2	Blood Volume for Full Assay
<0.5	>15 cc	>28 cc	>50 cc
0.5-1.0	15 cc	28 cc	50 cc
1.1-1.5	6.5 cc	12 cc	24 cc
1.6-2.0	4.5 cc	8.5 cc	16 cc
2.1-3.0	3.5 cc	6.5 cc	12 cc
3.1-4.0	2.5 cc	4.5 cc	8 cc
4.1-5.0	1.8 cc	3.5 cc	6 cc
>5.0	1.5 cc	2.5 cc	5 cc

### Specimen Minimum Volume

1 mL

**Reject Due To**

Gross hemolysis	Reject
Gross lipemia	OK

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
WB Sodium Heparin	Ambient	48 hours	GREEN TOP/HEP

**Clinical and Interpretive**
**Clinical Information**

T-cell stimulation in vitro is used extensively in the diagnostic immunology arena for facilitating T-cell proliferation and evaluation of T-cell function in a variety of clinical contexts.(1,2) The widely used method for assessing lymphocyte proliferation has been the measurement of 3H-thymidine incorporated into the DNA of proliferating cells. The disadvantages with the 3H-thymidine method of lymphocyte proliferation are: 1) the technique is cumbersome due to the use of radioactivity; 2) it does not allow discrimination of responding cell populations in response to stimulation; and 3) it does not provide any information on the contribution of apoptosis or cell death to the interpretation of the final result. Further, decreased lymphocyte proliferation could be due to several factors, including overall diminution of T-cell proliferation, decrease in proliferation of only a subset of T cells, or an apparent decrease in total lymphocyte proliferation due to T-cell lymphopenia and underrepresentation of T cells in the peripheral blood mononuclear cell pool. None of these can be discriminated by the thymidine uptake assay, but can be assessed by flow cytometry, which uses antibodies to identify specific responder cell populations. Cell viability can also be measured within the same assay without requiring additional cell manipulation or sample.

While mitogens such as phytohemagglutinin (PHA) activate T cells by binding to cell membrane glycoproteins, including the T-cell receptor (TCR)-CD3 complex, there are a number of mitogenic or comitogenic antibodies, including those directed against the CD3 coreceptor that can stimulate T-cell proliferation. Typically, anti-CD3 antibodies provide an initial activation signal, but do not induce significant proliferation, and the addition of a costimulatory antibody (anti-CD28) provides the stimulus for robust proliferation.(3) An exogenous T-cell growth factor, such as interleukin-2 (IL-2), may also be used as an alternate to anti-CD28 costimulation, and in patients with suspected IL-2 receptor-associated signaling defects, it may be more helpful than the use of anti-CD28. IL-2, an autocrine cytokine, has been demonstrated to be critical in T-cell proliferation.(4,5) The interaction of IL-2 with the IL-2 receptor (IL-2R) plays a central role in regulation of T-cell proliferation.(4) Triggering of the TCR leads to synthesis of IL-2 in certain T-cell subsets and induction of high-affinity IL-2Rs in antigen- or mitogen-activated T cells, and the binding of IL-2 to IL-2R ultimately leads to T-cell proliferation. The use of exogenous IL-2 in association with anti-CD3 allows discrimination of whether T cells, which cannot proliferate to other mitogenic signals, can respond to a potent growth factor such as IL-2. Stimulation of T cells with soluble antibodies to anti-CD3 (and the associated TCR complex) causes mobilization of cytoplasmic calcium and translocation of protein kinase C from the cytoplasm to the cell membrane. This stimulation also causes induction of phosphatidylinositol metabolism and subsequent IL-2 production for proliferation.(6) T-cell activation induced by anti-CD3 antibody requires prolonged stimulation of protein kinase C, which apparently can be achieved by the concomitant use of the anti-CD28 antibody for costimulation without addition of other mitogenic stimuli, such as phorbol myristate acetate (PMA).(7)

This assay uses a method that directly measures the S-phase proliferation of lymphocytes through the use of click chemistry. In the Invitrogen Click-iT-EdU assay, the click chemistry has been adapted to measure cell proliferation

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through direct detection of nucleotide incorporation. The Click-iT-EdU assay has been shown to be an acceptable alternative to the 3H-thymidine assay for measuring lymphocyte/T-cell proliferation.(8)

In the assay, an alkyne-modified nucleoside is supplied in cell-growth media for a defined time period and is incorporated within cells. The cells are subsequently fixed, permeabilized, and reacted with a dye-labeled azide, catalyzed by copper. A covalent bond is formed between the dye and the incorporated nucleotide, and the fluorescent signal is then measured by flow cytometry.(9) Specific proliferating cell populations can be visualized by the addition of cell-specific antibodies. Cell viability, apoptosis, and death can also be measured by flow cytometry using 7-aminoactinomycin D (7-AAD) and annexin V.

The absolute counts of lymphocyte subsets are known to be influenced by a variety of biological factors, including hormones, the environment, and temperature. The studies on diurnal (circadian) variation in lymphocyte counts have demonstrated progressive increase in CD4 T-cell count throughout the day, while CD8 T cells and CD19+ B cells increase between 8:30 am and noon, with no change between noon and afternoon. Natural killer (NK)-cell counts, on the other hand, is constant throughout the day. Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration. In fact, cortisol and catecholamine concentrations control distribution and, therefore, the numbers of naive versus effector CD4 and CD8 T cells. It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening, and during summer compared to winter. These data, therefore, indicate that timing, and consistency in timing, of blood collection is critical when serially monitoring patients for lymphocyte subsets.

### Reference Values

Viability of lymphocytes at day 0: > or =75.0%

Maximum proliferation of anti-CD3 as % CD45: > or =19.4%

Maximum proliferation of anti-CD3 as % CD3: > or =20.3%

Maximum proliferation of anti-CD3 + anti-CD28 as % CD45: > or =37.5%

Maximum proliferation of anti-CD3 + anti-CD28 as % CD3: > or =44.6%

Maximum proliferation of anti-CD3 + IL-2 as % CD45: > or =41.7%

Maximum proliferation of anti-CD3 + IL-2 as % CD3: > or =46.2%

### Interpretation

Abnormal test results to anti-CD3/aCD28/interleukin-2 (IL-2) stimulation are indicative of impaired T-cell function if T-cell counts are normal or only modestly decreased. If there is profound T-cell lymphopenia, it must be kept in mind that there could be a "dilution" effect with underrepresentation of T cells within the peripheral blood mononuclear cells (PBMC) population that could result in lower T-cell proliferative responses. However, this is not a significant concern in the flow cytometry assay, since acquisition of additional cellular events during analysis can compensate for artificial reduction in proliferation due to lower T-cell counts. The evaluation of T-cell proliferation to anti-CD3/IL-2 is likely to be helpful in assessing T-cell function in patients with refractory responses to other mitogenic and antigenic stimuli, specifically in the context of IL-2-receptor signaling defects, enabling greater mechanistic insight into the origins of T-cell dysfunction.

There is no absolute correlation between T-cell proliferation in vitro and a clinically significant immunodeficiency, whether primary or secondary, since T-cell proliferation in response to activation is necessary, but not sufficient, for an effective immune response. Therefore, the proliferative response to any mitogenic stimulus, including anti-CD3/anti-CD28, can be regarded as a more specific but less sensitive test for the diagnosis of infection susceptibility. It should also be kept in mind that there is no single laboratory test that can identify or define impaired cellular

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immunity, with the exception of identification of an opportunistic infection.

Controls in this laboratory and most clinical laboratories are healthy adults. Since this test is used for screening and evaluating cellular immune dysfunction in infants and children, it is reasonable to question the comparability of proliferative responses between healthy infants, children, and adults. One study has reported that the highest mitogen responses are seen in newborn infants with subsequent decline to 6 months of age, and a continuing decline through adolescence to half the neonatal response.<sup>(10)</sup> In our evaluation of 43 pediatric samples (of all ages) with adult normal controls, only 21% and 14% were below the tenth percentile of the adult reference range for the mitogens, pokeweed mitogen (PWM) and phytohemagglutinin (PHA), respectively. Comparisons between pediatric and adult data have not been performed for anti-CD3/aCD28 due to unavailability of prospective blood samples from healthy or patient pediatric donors for purposes of analytical validation.

It should be noted that without obtaining formal pediatric reference values it remains a possibility that the response in infants and children can be underestimated. However, the practical challenges of generating a pediatric range for this assay necessitate comparison of pediatric data with adult reference values or controls.

Lymphocyte proliferation responses to mitogens (including anti-CD3 stimulation) and antigens are significantly affected by time elapsed since blood collection. Results have been shown to be variable for specimens assessed greater than 24 and less than 48 hours post blood collection; therefore, lymphocyte proliferation results must be interpreted with due caution and results should be correlated with clinical context.

### **Cautions**

When interpreting results it should be kept in mind that the range of lymphocyte proliferative responses observed in healthy, immunologically competent individuals is large. The reference ranges provided (based on healthy donors) will be helpful in ascertaining the magnitude of the patient response.

Lymphocyte proliferation to mitogens is known to be affected by concomitant use of steroids, immunosuppressive agents, including cyclosporine (CsA), tacrolimus (FK506), CellCept (mycophenolate mofetil), immunomodulatory agents, alcohol, and physiological and social stress. Calcineurin inhibitors (CNI), such as CsA and tacrolimus would specifically inhibit interleukin-2 (IL-2)-stimulated proliferation since they specifically block production of IL-2 after T-cell activation. Also, IL-2-receptor-targeting monoclonal antibodies, such as daclizumab and basiliximab will also interfere with IL-2-stimulated proliferation.

Anti-CD3 proliferation should not be used as a first-line test when assessing lymphocyte proliferative responses. Ideally, mitogen proliferation (especially to phytohemagglutinin) should be assessed first, followed by antigens. If there are abnormal results to either, especially the former, it would be worthwhile to pursue additional testing for lymphocyte proliferation to anti-CD3 panel (as detailed above).

Test specimens greater than 24-hours old may give spurious results.

Diminished results may be obtained in specimens that contain excess neutrophils or nonviable cells.<sup>(11)</sup>

### **Clinical Reference**

1. Dupont B, Good RA: Lymphocyte transformation in vitro in patients with immunodeficiency diseases: use in diagnosis, histocompatibility testing and monitoring treatment. Birth Defects Orig Artic Ser 1975;11:477-485
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5. Cantrell DA, Smith KA: The interleukin-2 T cell system: a new cell growth model. *Science* 1984;224:1212
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8. Yu Y, Arora A, Min W, et al: EdU-Click iT flow cytometry assay as an alternative to 3H-thymidine for measuring proliferation of human and mice lymphocytes. *J Allergy Clin Immunol* 2009;123(2):S87
9. Salic A, Mitchison TJ: A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc Natl Acad Sci USA* 2008;105:2415-2420
10. Hicks MJ, Jones JK, Thies AC, et al: Age-related changes in mitogen-induced lymphocyte function from birth to old age. *Am J Clin Pathol* 1983;80:159-163
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## Performance

### Method Description

Peripheral blood mononuclear cells in RPMI 1640 medium supplemented with L-glutamine and 5% human AB serum are incubated unstimulated or stimulated with varying concentrations of anti-CD3, anti-CD3+anti-CD28, or anti-CD3+IL-2. A daily experimental normal control is included with each batch of patient samples to serve as an internal control.

Following incubation, the cells are assessed for proliferation and stained with the following markers: CD45+ lymphocytes, CD3+ T cells, and CD69+ activated cells. Results are reported for the percent viable cells on day 0, as well as the percentage of proliferating cells of total lymphocytes and T cells. (Package insert: 4, Invitrogen Click-iT-EdU; Unpublished Mayo method)

### PDF Report

No

### Day(s) Performed

Monday through Friday

Specimens are required to be received in the lab weekdays and by 4 p.m. on Friday. **No weekend processing.**

### Report Available

5 to 8 days

### Specimen Retention Time

No specimen retention. Entire specimen is used in preparation of the assay.

**Performing Laboratory Location**

Rochester

**Fees and Codes**
**Fees**

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

**CPT Code Information**

86353 x 1-Anti-CD3 + anti-CD28 stimulation

86353 x 1-Anti-CD3 + IL2 stimulation

86353 x 1-Anti-CD3 stimulation (as indicated)

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
LPA3P	Lymphocyte Proliferation, aCD3	59063-8

Result ID	Test Result Name	Result LOINC Value
35203	Viab of Lymphs at Day 0	33193-4
35171	Max Prolif, soluble aCD3 as % CD45	81760-1
35172	Max Prolif, soluble aCD3 as % CD3	81756-9
35173	Max Prolif, soluble aCD28 as % CD45	81759-3
35174	Max Prolif, soluble aCD28 as % CD3	81758-5
35176	Max Prolif, soluble IL2 as % CD45	81755-1
35177	Max Prolif, soluble IL2 as % CD3	81757-7
35205	Interpretation	69965-2
35204	aCD3 Comment	48767-8