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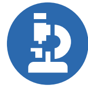
Produktinformation

Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

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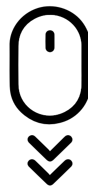
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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SZABO-SCANDIC HandelsgmbH
Quellenstraße 110, A-1100 Wien
T. +43(0)1 489 3961-0
F. +43(0)1 489 3961-7
mail@szabo-scandic.com
www.szabo-scandic.com

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Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
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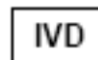

Product: FITC Anti-Human CD38
Cat. Ref: 38F-100T
Reagent provided: 100 test (20µl / test)
Description: Mouse Monoclonal Anti-Human CD38. Mouse Monoclonal Anti-Human CD38 FITC is recommended for use in flow cytometry for identification of Plasma cells. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide.
Clone: GR7A4
Isotype: IgG1
Fluorochrome: Fluorescein isothiocyanate, FITC (Ex-Max: 494 nm/Em-Max: 520 nm). Recommended 488 nm ion argon laser, 502 LP filter and 530/30 detector-equipped flow cytometer.).

INTENDED PURPOSE.

Immunostep's CD38 FITC is fluorochrome conjugated monoclonal antibody reagents. CD38 is a novel multifunctional ectoenzyme widely expressed in cells and tissues especially in Plasma Cells. CD38 also functions in cell adhesion,signal transduction and calcium signalling.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD38-antigen, which is strongly expressed plasma cell. The CD38-antigen is expressed at variable levels on the majority of hemopoietic cells, prevalently during early differentiation and activation. CD38 is expressed at variable levels on the majority of hemopoietic cells, prevalently during early differentiation and activation, especially at high levels on plasma cells. Constitutively is expressed also in brain, muscle, kidney and other tissues.

Specificity: Reacts with the 45 kDa type II single chain transmembrana glycoprotein, the cellular function is positive and negative regulator of cell activation and proliferation, depending on the cellular environment and it is involved in adhesion between human lymphocytes and endothelial cells.

CLINICAL RELEVANCE

- Leukemia phenotyping and classification
- Diagnosis and monitoring of multiple myeloma
- Monitoring of HIV-1 infection and progression
- Targeting of immunotoxin antibody in the treatment of myeloma
- Presence of autoantibodies with anti-CD38 specificity in patients with type II diabetes mellitus
- In mice, T-independent immune responses

PRINCIPLES OF THE TEST.

Immunostep CD38: FITC, monoclonal antibodies bind to the surface of cells that express the CD38 antigen. To identify these cells, peripheral blood leucocytes are incubated with the antibodies and red blood cells are lysed before washing to remove unbound antibodies. An appropriate fixative solution is added to lysed, washed cells before the stained and fixed cells are analysed by flow cytometry with an argon ion laser at 488nm.

REAGENTS.

Cluster Designation:	CD38
WHO Classification:	Leukocyte Workshop IV.
Clone:	GR7A4
Isotype:	IgG1
Species:	Mouse
Composition:	IgG1 heavy chain Kappa light chain
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	Fluorescein (FITC) Excitation wavelength 488 nm Emission wavelength 520 nm
Molar composition:	FITC/protein 6,0 – 8,0

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Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. / Fax: (+34) 923 294 827
E-mail: info@immunostep.com
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Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests, the conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽0.09% sodium Azide
Reagent preparation:	Ready to use.

1. STATEMENTS, SETTINGS AND WARNINGS.

Reagents contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Light exposure should be avoided. Use dim light during handling, incubation with cells and prior to analysis.

Do not pipet by mouth.

Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.

The sample preparation procedure employs a fixative (formaldehyde). Contact is to be avoided with skin or mucous membranes.

Do not use antibodies beyond the stated expiration dates of the products.

Deviations from the recommended procedure enclosed within this product insert may invalidate the results of testing.

FOR IN VITRO DIAGNOSTIC USE

For professional use only.

2. APPROPIATE STORAGE CONDITIONS.

Fluorescein (FITC) Keep in dark place at 2-8ºC. DO NOT FREEZE.

**Note: it's been described stored conjugated monoclonal antibodies on FITC at -20ºC. This can affect to the conjugated intense.*

3. EVIDENCE OF DETERIORATION.

Reagents should not be used if any evidence of deterioration or substantial loss of reactivity is observed. For more information, please contact with our technical service: tech@immunostep.com

- The normal appearance of the FITC conjugated monoclonal antibody is a clear, yellow-orange liquid.

4. SPECIMEN COLLECTION.

Collect venous blood samples into blood collection tubes using an appropriate anticoagulant (EDTA or heparin). For optimal results the sample should be processed within 6 hours of venipuncture. EDTA, ACD or heparin may be used if the blood sample is processed for analysis within 30 hours of venipuncture. ACD or heparin, but not EDTA, may be used if the sample is not processed within 30 hours of venipuncture. Samples that cannot be processed within 48 hours should be discarded.

If venous blood samples are collected into ACD for flow cytometric analysis, a separate venous blood sample should be collected into EDTA if a CBC is required.

Unstained anticoagulated blood should be retained at 20- 25oC prior to sample processing. Blood samples that are hemolyzed, clotted or appear to be lipemic, discoloured or to contain interfering substances should be discarded.

Refer to "*Standard Procedures for the Collection of Diagnostic Blood Specimens*" published by the National Committee for Clinical Laboratory Standards (NCCLS) for additional information on the collection of blood specimens.

5. SAMPLE PREPARATION.

1. Add 20 µL of CD38 FITC and mix gently with a vortex mixer. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory
2. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube (10^6 cells).
3. The recommended negative control is a non-reactive FITC-conjugated antibody of the same isotype.
4. Incubate in the dark at room temperature (20-25 °C) for 15 minutes or at 4 °C for 30 minutes.
5. Add Lysing Solution according to the manufacturer's directions to each sample and mix gently with a vortex mixer.

Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. / Fax: (+34) 923 294 827
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6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.
7. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
8. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
9. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS + 0,5 % BSA.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis.

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents: Mouse IgG1: FITC

Leucocyte gating reagent: Mouse anti-human CD45: PE/CD14 PE

Serofuge or equivalent centrifuge

12 x 75 mm polypropylene centrifuge tubes

Micropipette capable of dispensing 5 µl, 20 µl, 100 µl, and 500 µl volumes

Blood collection tubes with anticoagulant

Phosphate buffered saline (PBS)

Trypan Blue or propidium iodide, 0.25% (w/v) in PBS for the determination of cell viability

Lysing Solution

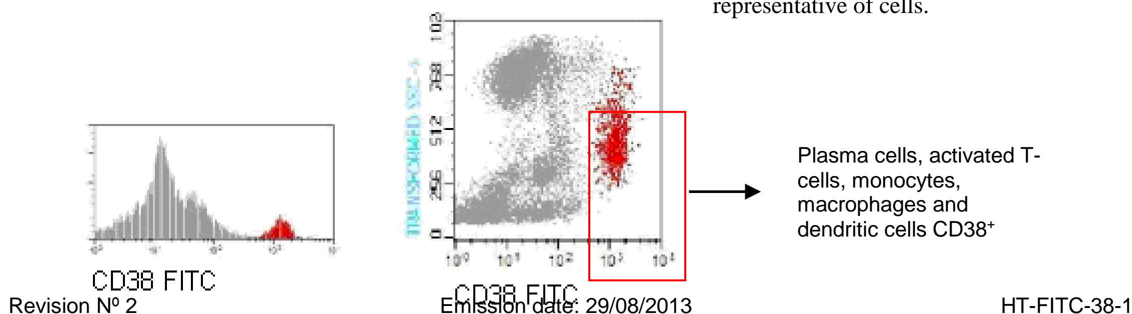
Fixing Solution

Flow cytometer: Becton Dickinson FACScan™, Coulter Profile or equivalent.

7. INTERPRETATION OF RESULTS.

FLOW CYTOMETRY

Analyze antibody-stained cells on an appropriate flow cytometer analyzer according to the manufacturer instructions. The right angle light scatter or other scatter (SSC) versus forward angle light scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the lymphocyte cluster (lymphocyte bitmap). The fluorescence attributable to the FITC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained T lymphocytes is determined. An appropriate FITC- conjugated isotypic control of the same heavy chain immunoglobulin class and antibody concentration must be used to estimate and correct for non-specific binding to lymphocytes. An analysis region is set to exclude background fluorescence and to include positively stained cells. The following histograms are representative of cells.



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Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
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Tel. / Fax: (+34) 923 294 827
E-mail: info@immunostep.com
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Cells were analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer, using Cell Quest acquisition software and PAINT-A-GATE. PRO, analysis software.

8. QUALITY CONTROL PROCEDURES.

Non-specific fluorescence identified by the FITC conjugated isotypic control is usually less than 2% in normal individuals. Non-specific fluorescence identified by the PE and APC conjugated isotypic controls are usually less than 4% in normal individuals. If the background level exceeds these values, test results may be in error. Increased non-specific fluorescence may be seen in some disease states.

A blood sample from each normal and abnormal donor should be stained with the CD45 Pan-lymphocyte and CD14 Pan-monocyte monoclonal antibodies. When used in combination, these reagents assist in identifying the lymphocyte analysis region, and distinguish lymphocytes from monocytes, granulocytes and unlysed or nucleated red cells and cellular debris.

A blood sample from a healthy normal donor should be analyzed as a positive control on a daily basis or as frequently as needed to ensure proper laboratory working conditions. Each laboratory should establish their own normal ranges, since values obtained from normal samples may vary from laboratory to laboratory.

An appropriate isotype control should be used as a negative control with each patient sample to identify non-specific Fc binding to lymphocytes. An analysis region should be set to exclude the non-specific fluorescence identified by the isotypic control, and to include the brighter fluorescence of the lymphocyte population that is identified by the specific antibody.

Refer to the appropriate flow cytometer instrument manuals and other available references for recommended instrument calibration procedures.

9. LIMITATIONS OF THE PROCEDURE.

1. Incubation of antibody with cells for other than the recommended time and temperature may result in capping or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.
3. Abnormal cells or cell lines may have a higher antigen density than normal cells. This could, in some cases, require the use of a larger quantity of monoclonal antibody than is indicated in the procedures for Sample Preparation.
4. Blood samples from abnormal donors may not always show abnormal values for the percentage of lymphocytes stained with a given monoclonal antibody. Results obtained by flow cytometric analysis should be considered in combination with results from other diagnostic procedures.
5. When using the whole blood method, red blood cells found in some abnormal donors, as well as nucleated red cells found in normal and abnormal donors may be resistant to lysis by lysing solutions. Longer red cell lysis periods may be needed to avoid the inclusion of unlysed red cells in the lymphocyte gated region.
6. Blood samples should not be refrigerated or retained at ambient temperature for an extensive period (longer than 24-30 hours) prior to incubating with monoclonal antibodies.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the laser, as well as proper gate settings.
8. Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary.
9. Al results need to be interpreted in the context of clinical features, complete immunophenotype and cell morphology, taking due account of samples containing a mixture of normal and neoplastic cells.

10. REFERENCE VALUES.

The cellular elements of human Bone Marrow include lymphocytes, monocytes, granulocytes, red blood cells and platelets.

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Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. / Fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com

Nucleated cells Percentage in the Bone Marrow

Cell type	Percentage
Progranulocytes	56,7
Neutrophils	53,6
Myeloblasts	0,9
Promyeloblasts	3,3
Promyelocytes	12,7
Metamyelocytes	15,9
Eosinophils	3,1
Basophils	<0,1
Proerythrocyte	25,6
Proerythrblasts	0,6
Basophil Erythroblast	1,4
Polycromatic Erythroblast	21,6
Ortocromatic Erythroblast	2
Megakaryocytes	<0,2
Lymphocytes	16,2
Plasma cells	2,3
Reticular cells	0,4

Normal human peripheral blood lymphocytes 20-47% (n=150% confidence interval)

Nucleated cells Percentage in Peripheral Blood of a Normal Patient

Cell type	Percentage	Number of event.
Red Blood Count		3,8 - 5,6 $X10^6/\mu L$
Platelets		150 - 450 $X10^3/\mu L$
White Blood Count (WBC)		4.3 - 10.0 $X10^3/\mu L$
Neutrophils	57 – 67 %	1,5 - 7.0 $X10^3/\mu L$
Lymphocytes*	25 – 33 %	1.0 - 4.8 $X10^3/\mu L$
T cell	56 – 82 % of lymphocytes	
T cell CD4+	60 % of T cells	
T cell CD8+	40 % of T cells	
Cell NK+	6 – 33 of lymphocytes	
B cell	7.7 – 22 of lymphocytes	
Monocytes	3 – 7 %	0.28 - 0.8 $X10^3/\mu L$
Eosinophils	1 – 3 %	0.05 – 0,25 $X10^3/\mu L$
Basophils	0 – 0,075 %	0,015 – 0,05 $X10^3/\mu L$
Reticulocyte	0,5 – 1,5 % of total Red Blood Cell	

Expected values for pediatrics and adolescents have not been established.

The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.

11. PERFORMANCE CHARACTERISTICS.

SPECIFICITY

Blood samples were obtained from healthy normal donors of Caucasian were stained with Immunostep CD38 FITC monoclonal antibody. Cells contained in the lymphocyte, monocyte and granulocyte regions were selected for analysis. Blood samples were processed by a leukocyte method, with a direct immunofluorescence staining for flow cytometric analysis.

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Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. / Fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com

To evaluate the reagent's Specificity (cross-reactivity with other cell populations), 10 blood samples from healthy donors were studied, stained with an adequate isotype control and the MAb to study. The percentage of lymphocytes, monocytes and granulocytes stained with the mentioned MAb was evaluated. The results obtained are shown in the following table:

Case Summaries

Sample	% Lymphocytes	% Monocytes	% Granulocytes
1	44,07	65,21	73,67
2	42,92	90,02	86,47
3	44,68	91,66	77,88
4	47,92	96,05	56,98
5	36,98	94,12	71,59
6	24,20	89,97	65,70
7	25,82	92,07	69,72
8	54,35	89,55	80,59
9	29,25	68,19	67,02
10	32,19	87,21	64,39
Total N	10	10	10

Statistics

		% Lymphocytes	% Monocytes	% Granulocytes
N	Valid	10	10	10
	Missing	0	0	0
Mean		38,2380	86,4050	71,4010
Median		39,9500	89,9950	70,6550
Mode		24,20	65,21	56,98
Std. Deviation		10,11593	10,69572	8,63014
Variance		102,332	114,398	74,479
Range		30,15	30,84	29,49
Minimum		24,20	65,21	56,98
Maximum		54,35	96,05	86,47

Multiple modes exist. The smallest value is shown

Address: Avda. Universidad de Coimba, s/n
Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
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E-mail: info@immunostep.com
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SENSIBILITY

Sensitivity of the Immunostep CD38 monoclonal antibodies was determined by staining a blood sample from donor. Dilutions of a peripheral blood sample were made to check the concentration scale of stained cells obtained. The results show an excellent correlation level between the results obtained and expected based on the dilution used.

To determine the consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate). It provides an indication of its reliability during its normal use.

Case Summaries

	Sample	Dilution	% Expected	% Obtained
1	400A + 0B	100,0	64,33	64,33
2	350A + 50B	87,5	56,28	54,60
3	300A + 100B	75,0	48,24	46,21
4	250A + 150B	62,5	40,20	39,91
5	200A + 200B	50,0	32,16	30,41
6	150A + 250B	37,5	24,12	22,54
7	100A + 300B	25,0	16,08	15,75
8	50A + 350B	12,5	8,04	10,15
9	0A + 400B	,0	,00	2,54
Total N	9	9	9	9

Model Summary

Model	R	R Square	Adjusted R Spuare	Std. Error of the Estimate
1	,998[a]	,996	,996	1,44559

(a) Predictors: (Constant), Obtained

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37007 Salamanca (Spain)
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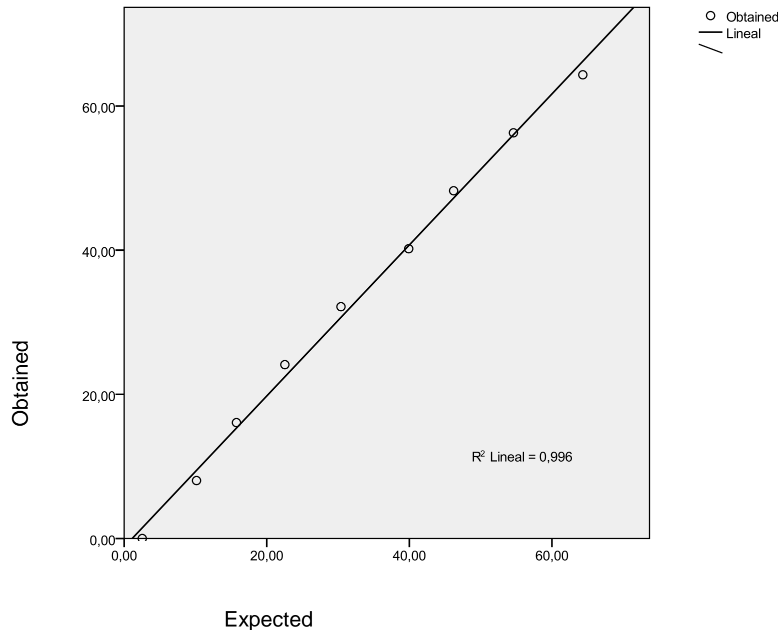

REPRODUCIBILITY

Reproducibility for the Immunostep CD38 FITC-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD38+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD38. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Lymphocytes were selected for the analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from a normal donor expressing a high percentage of CD38+ cells. Mid range and low range samples were obtained by mixing known CD38- cells in appropriate ratios, while maintaining the same total cell concentration for the three ranges.

The study was performed in each of three independent laboratories, in the manner that each laboratory obtained, stained and analyzed separate blood samples.

Case Summaries

	% High	% Medium	% Low
1	82,15	72,64	54,87
2	82,02	72,22	58,64
3	81,53	71,13	61,20
4	79,76	72,66	59,48
5	80,62	71,82	59,09
6	81,70	74,09	59,55
7	81,09	73,87	58,32
8	80,92	73,62	60,08
9	81,90	74,63	59,37

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Cancer Research Center (C.I.C.)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
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E-mail: info@immunostep.com
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10	82,59	61,91	58,72
Total N	10	10	10

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
% High	10	2,83	79,76	82,59	81,4280	,83908	,704
% Medium	10	12,72	61,91	74,63	71,8590	3,66210	13,411
% Low	10	6,33	54,87	61,20	58,9320	1,64538	2,707
Valid N (listwise	10						

***Note:** Data analyzed with SPSS for Windows 17.0*

12. BIBLIOGRAPHY.

1. Ferrero E; Malavasi F. The metamorphosis of a molecule: from soluble enzyme to the leukocyte receptor CD38. J. Leukoc. Biol. 1999 65:151 PubMed.

2. Lund F; Solvason N; Grimaldi JC; Parkhouse RM; Howard M. Murine CD38: an immunoregulatory ectoenzyme. Immunol. Today 1995 16:469 PubMed.

3. Malavasi F; Funaro A; Roggero S; Horenstein A; Calosso L; Mehta K. Human CD38: a glycoprotein in search of a function. Immunol. Today 1994 15:95 PubMed .

4. Mehta K; Shahid U; Malavasi F. Human CD38, a cell-surface protein with multiple functions. FASEB J. 1996 10:1408 PubMed .

5. Shubinsky G; Schlesinger M. The CD38 lymphocyte differentiation marker: new insight into its ectoenzymatic activity and its role as a signal transducer. Immunity 1997 7:315 PubMed