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A Brighter Future in Flow

Phagocytosis

Why to survey phagocytic activity?

To confirm or exclude defective phagocytosis, primary and secondary immunodeficiences.

When diagnosing primary immunodeficiences, the examination of phagocytic activity is the first method of choice! Clinical symptoms of hereditary immunodeficiences usually manifest in early childhood, however, the less severe forms can sometimes be manifested later in adulthood. Without early and adequate therapy, these illnesses are life threatening.

Primary immunodeficiences

- Chronic granulomatous disease (defective bactericidal mechanisms caused by a lack of NADPH oxidase function, incidence 1:200 000, clinically manifested by severe infections and granulomas in organs)
- Defective myeloperoxidase (results in frequent candidiasis, a relatively widespread defect)
- Glucose-6-phosphate dehydrogenase deficiency
- Defective glutathione synthetase (impaired regeneration of NADPH)
- Chédiak-Higashi syndrome (anomalous granula, impaired degranulation)
- Defective ingestion

Secondary immunodeficiences

These are caused by external agents, including medication.

A survey of phagocytic activity is recommended when monitoring the clinical state of a patient after medication by the following drugs:

- Corticosteroids and other immunosuppressants
- Cytostatics (cyclophosphamide and other myelotoxic drugs)
- Cytokines and growth factors (G-CSF, GM-CSF, IFN-γ, TNF)

A survey is also recommended:

- After transplantation of hematopoietic stem cells
- In cases of systemic inflammatory response syndrome (SIRS)

Decreased phagocytic activity reveals a high risk of infection leading to sepsis!

Phagocytosis, the process where specialised cells of the immune system kill and decompose microorganisms (e.g. extracellular bacteria), is fundamental to innate non-specific human immunity. Polymorphonuclear leukocytes (neutrophilic granulocytes), macrophages and dendritic cells are the effector cells in the process of phagocytosis.

Phagocytosis is preceded by chemotactic migration of the phagocytes (mainly neutrophilic granulocytes) into the site of inflammation. Then the foreign particles are recognized, ingested (forming phagosome and phagolysosome), and finally destructed by oxygen-independent and oxygen-dependent mechanisms. The latter ones (the “oxidative burst”) involve a release of reactive oxygen radicals, hydrogen peroxide, hydroxyl radical, and hypochlorite. In this process the key enzymes are the NADPH oxidase and myeloperoxidase, whose malfunction leads to impaired bactericidal mechanisms.

Examination of defective phagocytosis by flow cytometry

FagoFlowEx Kit (Cat. No.: ED7042)

The FagoFlowEx Kit is intended for examination of phagocytic activity of neutrophil granulocytes by measuring the respiratory (oxidative) burst after their stimulation with *E. coli* bacteria in human heparinized whole blood using flow cytometry.

IngoFlowEx Kit (Cat. No.: ED7040)

IngoFlowEx Kit is designed for quantification of phagocytic activity of human granulocytes and monocytes by measuring the ingestion of fluorescently labeled *E. coli* bacteria in human heparinized whole blood using flow cytometry.

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When to survey phagocytic activity?

The examination is recommended for people displaying the following symptoms:

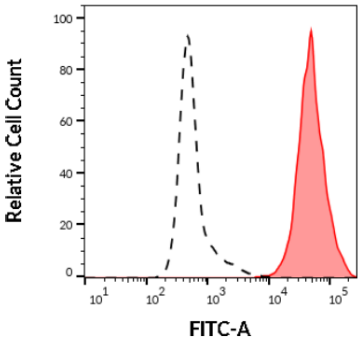
- Recurrent and chronic infections of the skin and mucosal surfaces (pyoderma, abscesses, impetiginised dermatitis, disseminated candidiasis, chronic furunculosis, localized ulcerous inflammation)
- Infections of the lymph nodes
- Gingivitis, aphthous stomatitis
- Infections of respiratory tract (otitis, sinusitis, bronchitis)
- Pelvic inflammatory disease
- Osteomyelitis, meningitis
- Sepsis
- BCG-itis after TBC vaccination
- In cases where infections are caused by pyogenic bacteria, fungi and parasites, and predominantly by *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Streptococcus pneumoniae, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Mycobacterium* spp. incl. BCG, *Listeria spp., Salmonella spp., Nocardia spp., Candida spp., Aspergillus fumigatus, Toxoplasma gondii, Pneumocystis carinii, Pneumocystis jiroveci, Cryptococcus neoformans, Isospora belli, Microsporidium spp.* etc.

FagoFlowEx Kit

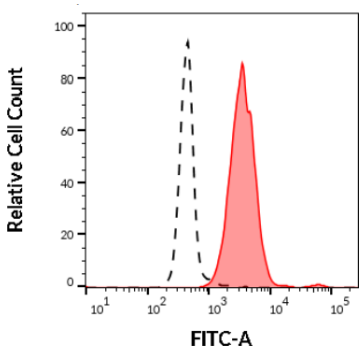
This test is based on the measurement of respiratory burst of neutrophil granulocytes after their stimulation with *E. coli* bacteria. During the process of bacteria ingestion, phagocytes activate the NADPH oxidase producing reactive oxidative intermediates (respiratory burst). Resulting hypochlorite ions inside phagocytes strongly oxidize added dihydrorhodamine 123 (DHR123) into fluorescent rhodamine 123 which is detected by a flow cytometer. In the case of MPO deficiency, DHR123 is oxidized with less intensity by other oxidative products resulting in lower fluorescence intensity of stimulated granulocytes. A positive control sample is stimulated using PMA (phorbol 12-myristate 13- acetate) which activates respiratory burst of granulocytes without adhesion and ingestion of the pathogen.

Reagents provided:

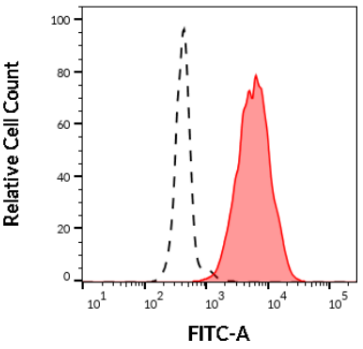
ED7042-1 *E.coli* – 5 vials containing lyophilized *E. coli* bacteria, 1 vial is intended for stimulation of 20 tubes.
ED7042-2 DHR123 – 5 vials containing lyophilized Dihydrorhodamine 123, 1 vial is intended for staining of 60 tubes.
ED7042-3 Stimulation Control – 5 vials containing lyophilized PMA (Phorbol 12-myristate 13-acetate), 1 vial is intended for stimulation of 20 positive controls.
ED7042-4 Lysing Solution – 15 ml



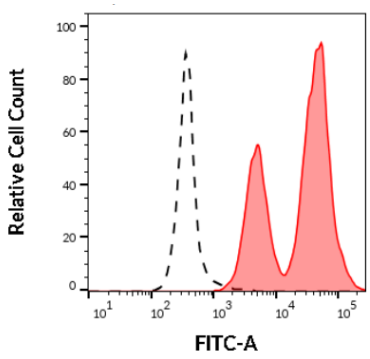
Histogram overlay: Healthy donor without defect of respiratory burst, (SI = 98).



Histogram overlay: Patient with MPO deficiency, (SI = 11).



Histogram overlay: Male patient with CGD, (SI = 16).



Histogram overlay: Female carrying X-linked mutation of the NADPH oxidase gene. Two granulocyte subpopulations differ in respiratory burst intensity, (SI^{low} = 13.9, SI^{high} = 125).

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Product	Cat. No.	Quantity	Reg. Status
FagoFlowEx Kit	ED7042	100 tests	CE IVD

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Other immunodeficiency products

https://www.exbio.cz/clinical-products/immunodeficiency

- Kits (6)
- COVID-19 kits (2)
- Antibody combinations (13)
- Premium antibodies (GMP) (183)
- Antibodies (17)
- Mitogens (5)
- Lysing solutions (4)

Other clinical products

https://www.exbio.cz/clinical-products

- PREMIUM ANTIBODIES
- IMMUNODEFICIENCY
- ALLERGY
- REPRODUCTIVE IMMUNOLOGY
- HEMATOLOGY
- NEW PRODUCTS

COVID-19 products

https://www.exbio.cz/clinical-products/immunodeficiency/covid-19-kits

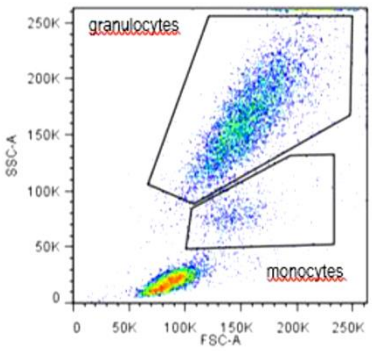
- DryFlowEx ACT T Screen Kit
- DryFlowEx ASC Screen Kit
- EXCELLYSE Easy

IngoFlowEx Kit

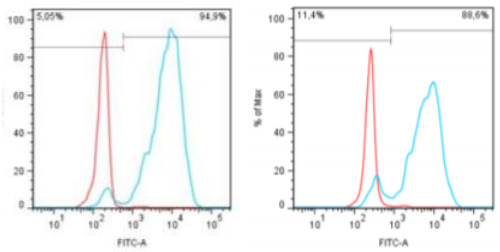
The test is based on measuring the fluorescence of cells that ingested FITC-labeled *E. coli*. A sample of heparinized blood is mixed with fluorescent *E. coli* and incubated at 37 °C. The control reaction without *E. coli* is performed in parallel with each reaction with *E. coli*. This negative control is used to set the discrimination boundery between the phagocyting and the nonphagocyting cells. The non-ingested bacteria are separated by repeated washes. The fluorescence of remaining extracellular and surface bound bacteria is quenched with trypan blue, a vital dye does not cross the cellular membrane. Samples are then subjected to erythrocyte lysis and fixed. Finally the cellular DNA is stained with propidium iodide. DNA staining helps to define nucleated cells from debris and clumps of bacteria. Bacteria used in the test were opsonised with human AB plasma, the results are not primarily dependent on opsonizing activity of the tested blood sample.

Reagents provided:

ED7040-1 *E. coli* FITC, 1 x 1 ml, intended for 100 tests.
ED7040-2 Quenching Solution, 1 x 20 ml, intended for 200 tests.
ED7040-3 Lysing Solution, 1 x 60 ml, intended to prepare 600 ml of 1x Lysing Solution = 300 tests.
ED7040-4 Wash Buffer, 1 x 80 ml, intended to prepare 2 liters of 1x Wash buffer = 222 tests.
ED7040-5 DNA Staining Solution, 1 x 60 ml,
intended for 200 tests.), 1 vial is intended for stimulation of 20 positive controls.
ED7042-4 Lysing Solution – 15 ml



Gates for granulocytes and monocytes.



Overlay of histograms of granulocyte and monocyte subpopulation.

Product	Cat. No.	Quantity	Reg. Status
IngoFlowEx Kit	ED7040	100 tests	RUO

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