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A Brighter Future in Flow

FagoFlowEx kit – Inovace a změny soupravy

10. 06. 2025, Pavel Jinoch

FagoFlowEx Kit, kat.č. ED7042



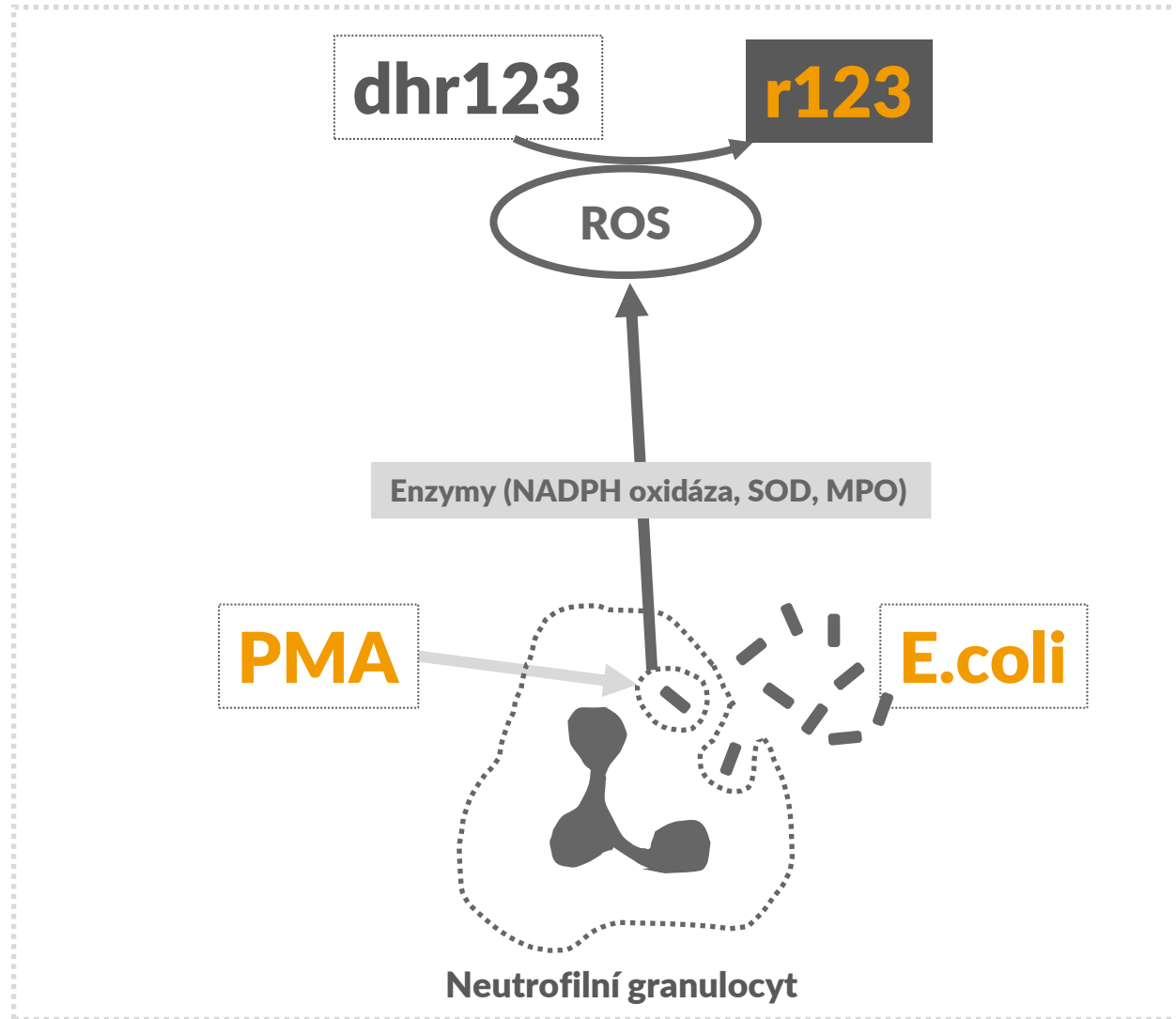
Funkční test fagocytů
pro průtokovou
cytometrii

Od roku 2012 certifikát
CE IVD

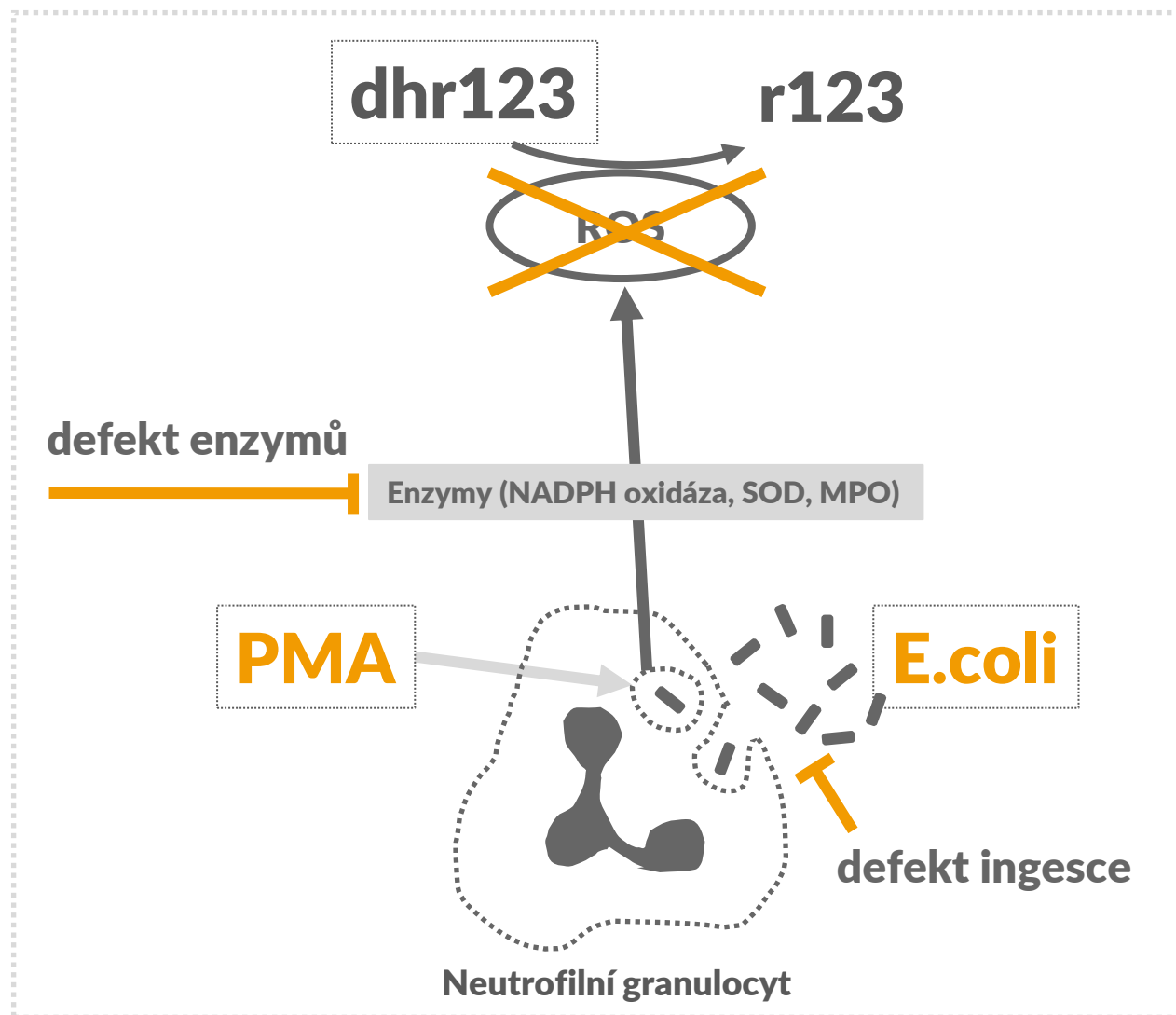
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Princip metody



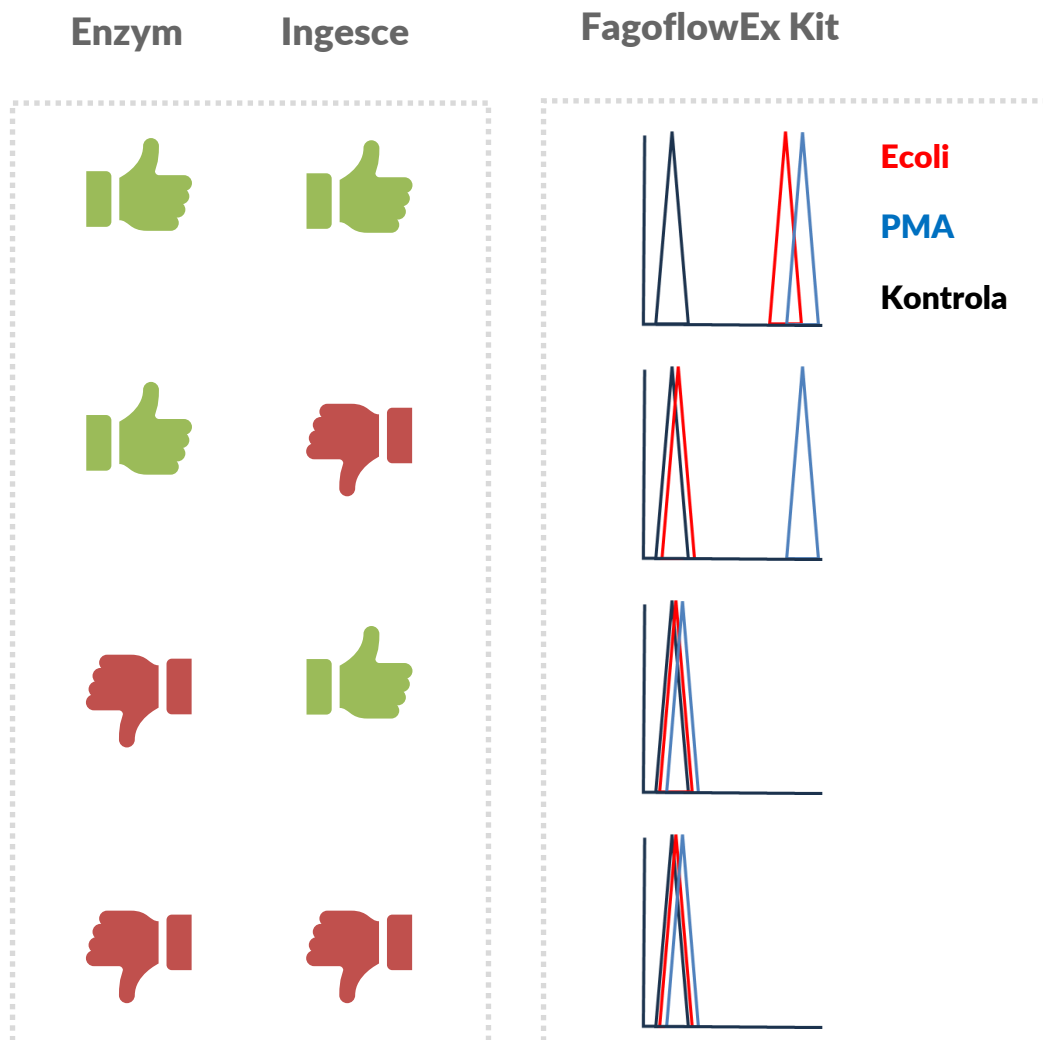
Princip metody



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Výsledek



% fagocytyjících

Relativní počet pozitivních granulocytů, které vykazovaly respirační vzplanutí po stimulaci E. coli.

Stimulační Index

Stimulační index (SI) počítaný jako poměr střední hodnoty fluorescence (MFI) pozitivních granulocytů z reakce stimulované E. coli a negativních granulocytů z negativní kontrolní reakce.

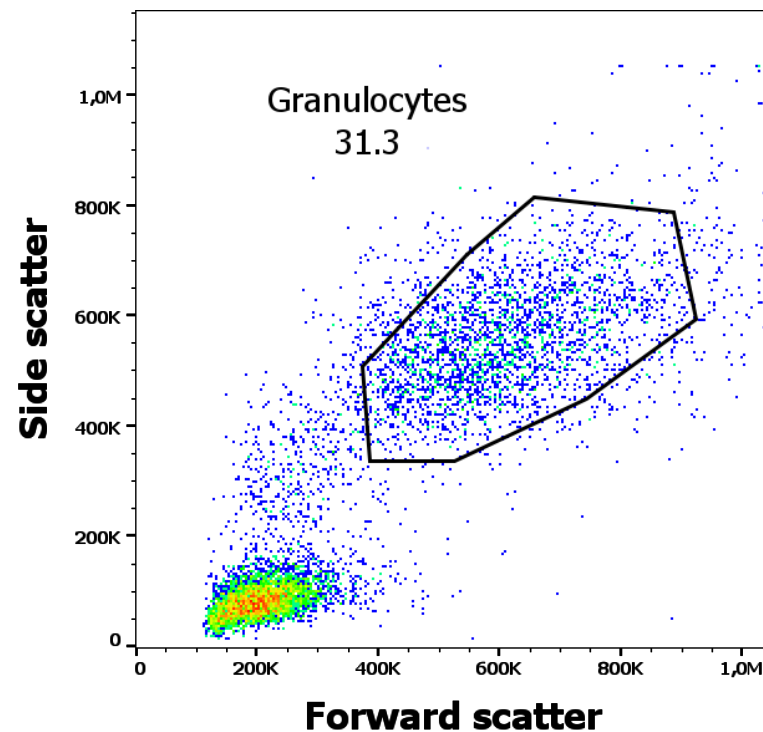
Určený účel použití

**Stanovení fagocytární aktivity
neutrofilních granulocytů měřením
respiračního (oxidačního) vzplanutí
z plné krve**

pomocí průtokové cytometrie



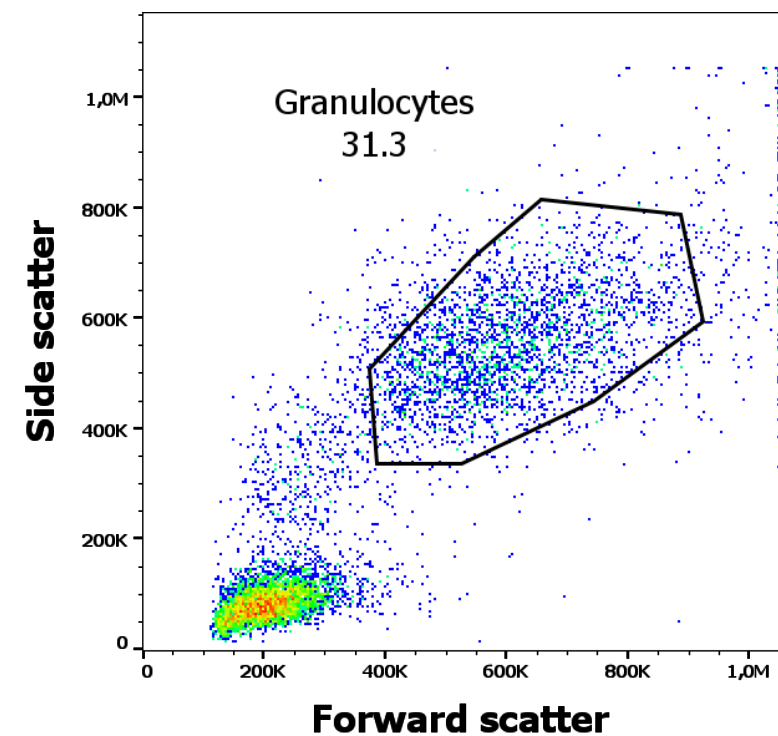
Vyšetření vzorku



Identifikace granulocytů: Optické scatterry

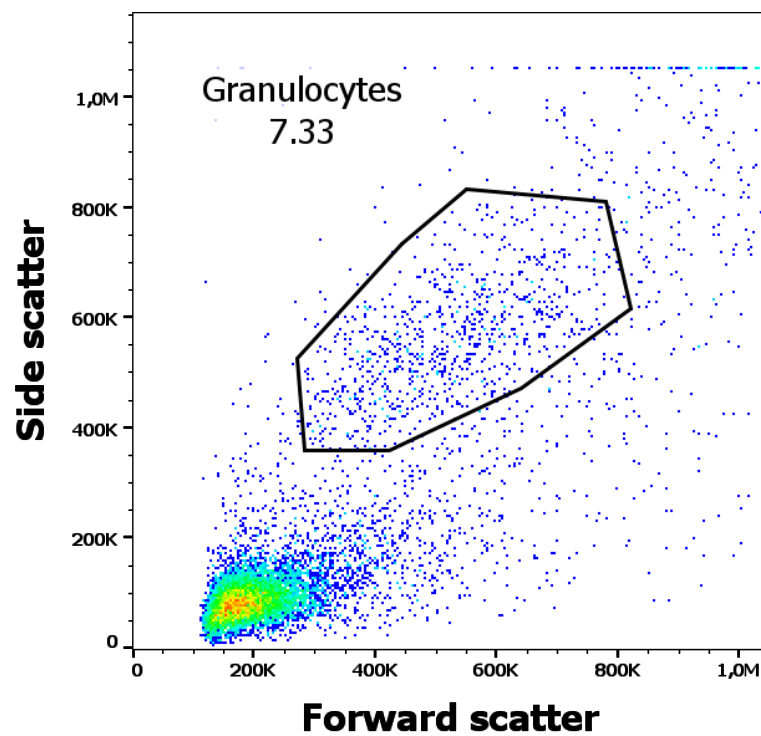
Rok 2012
Cytometer: Cytomics FC500

Kontrola



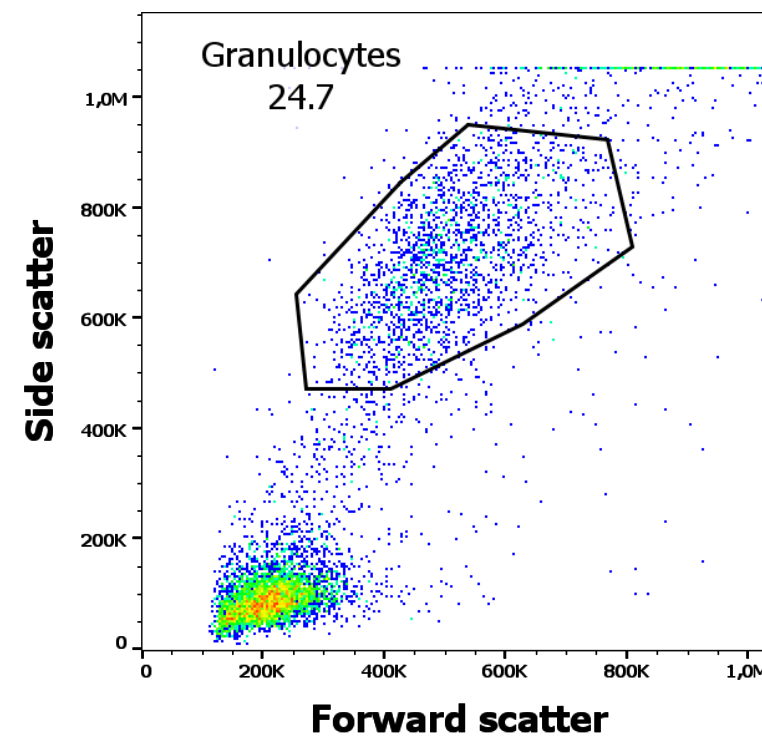
Počet granulocytů: 2701

PMA



Počet granulocytů: 872

E.coli

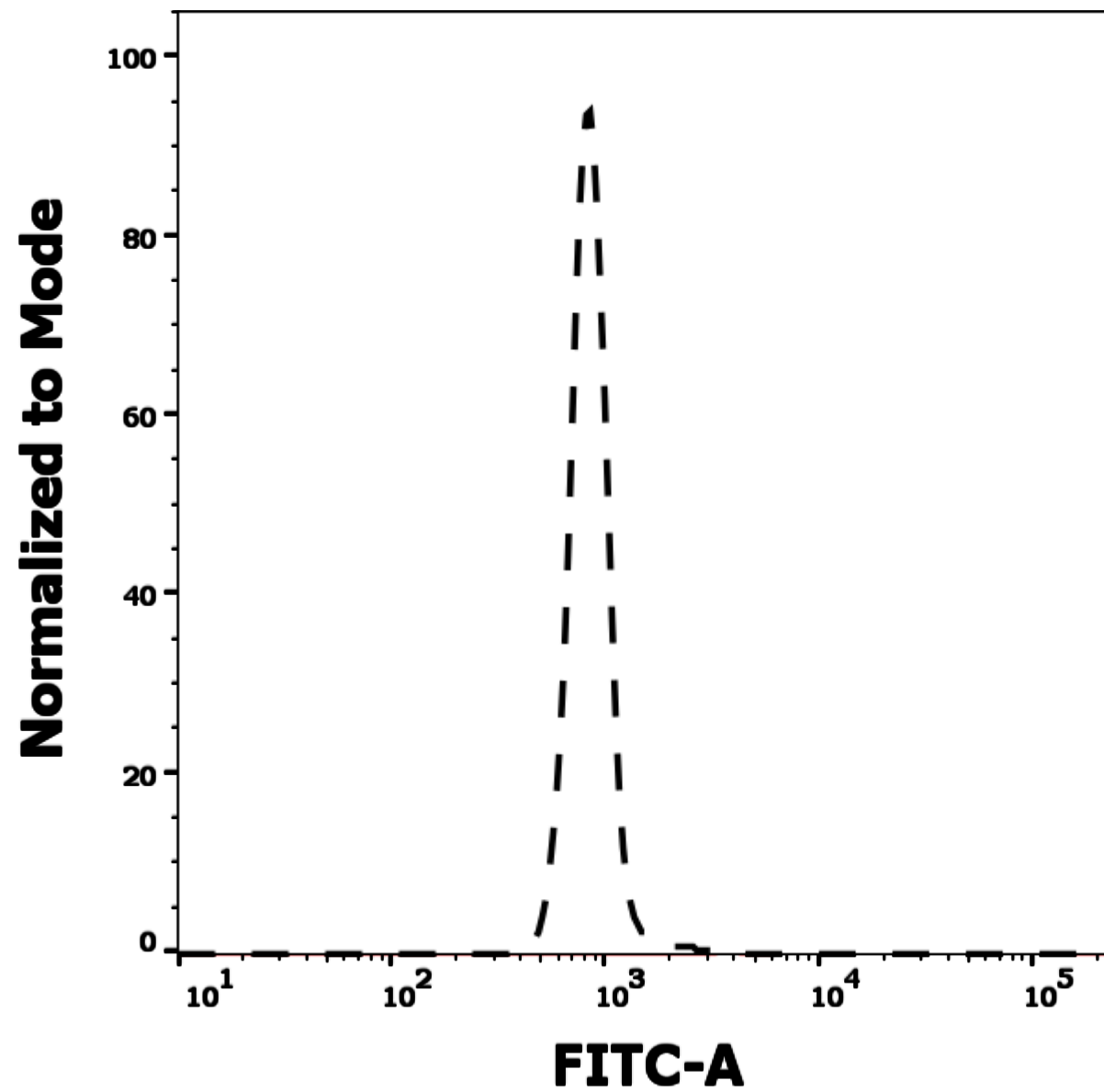


Počet granulocytů: 1979

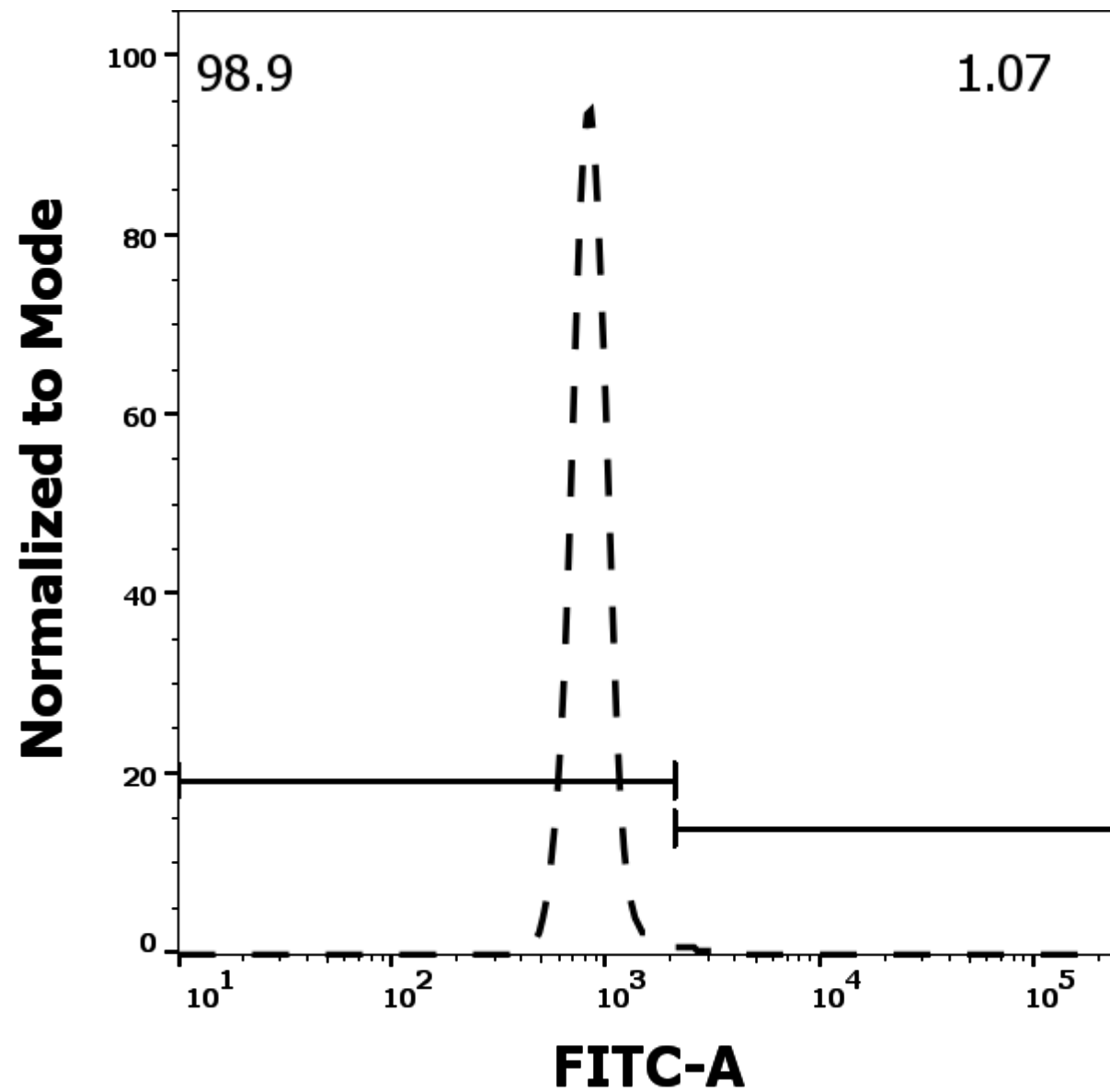
Rok 2012
Cytometer: Cytomics FC500



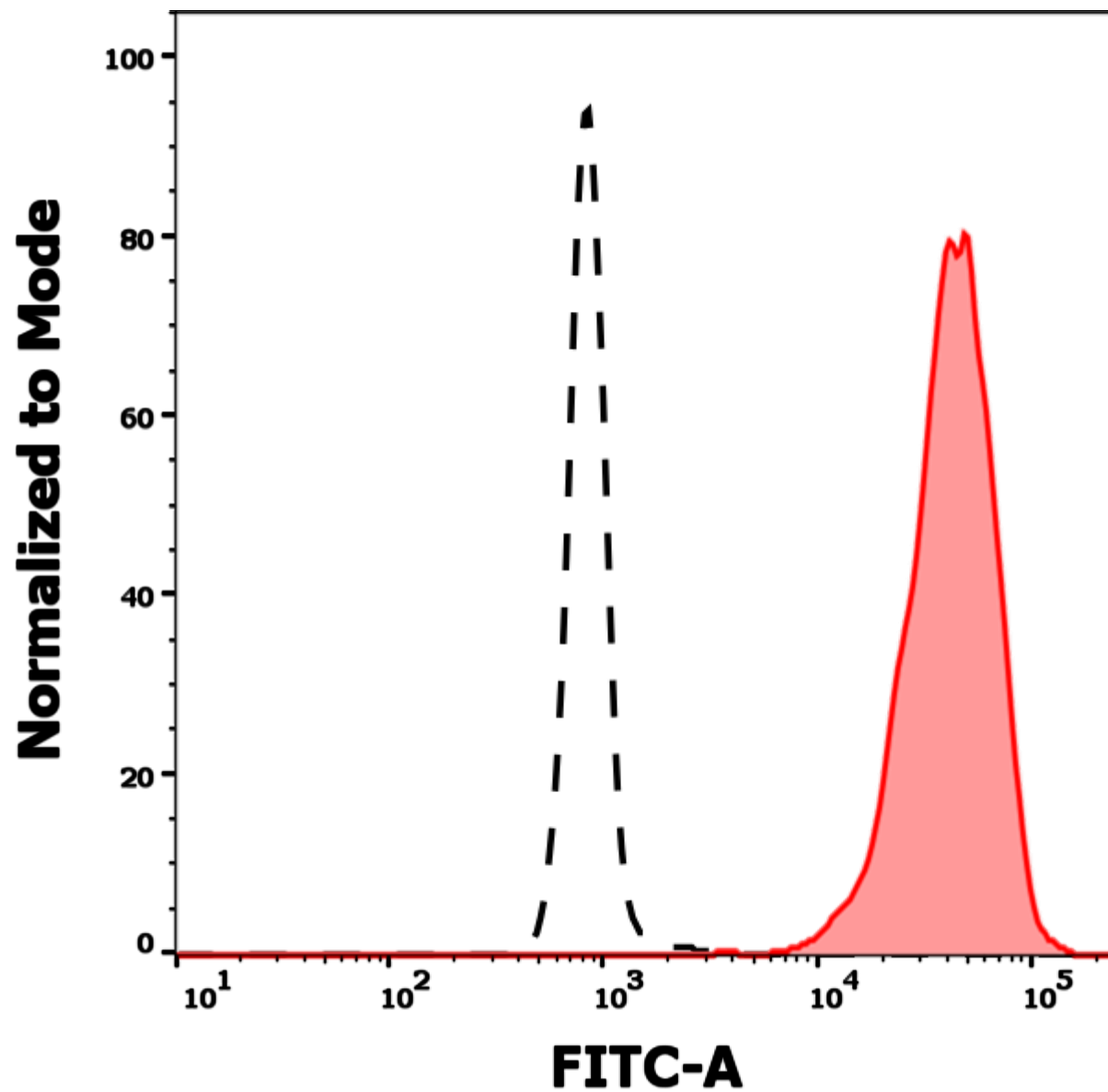
- **Kontrola**



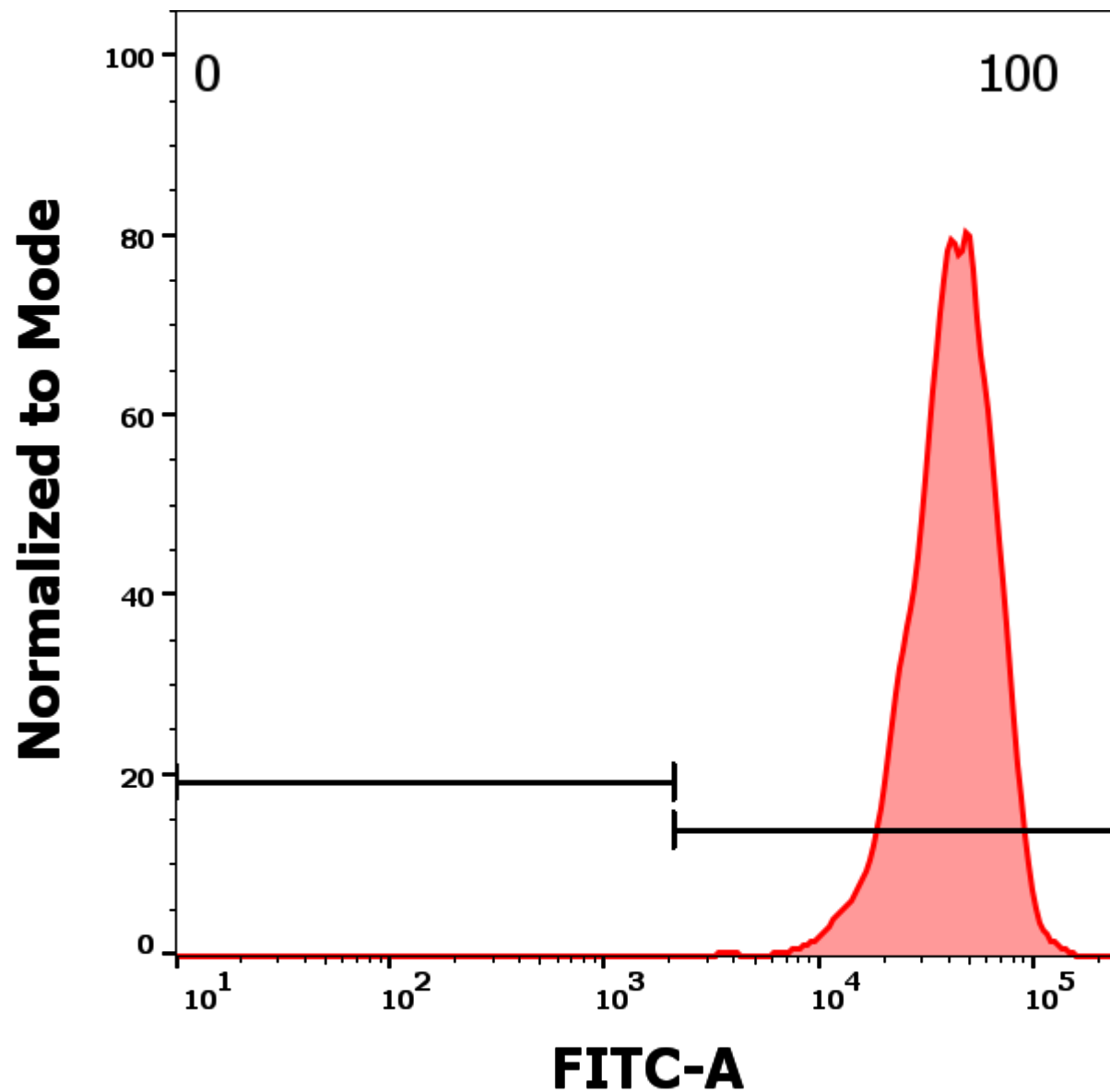
- **Kontrola**
- **Rozdělení na nestimulované a stimulované**



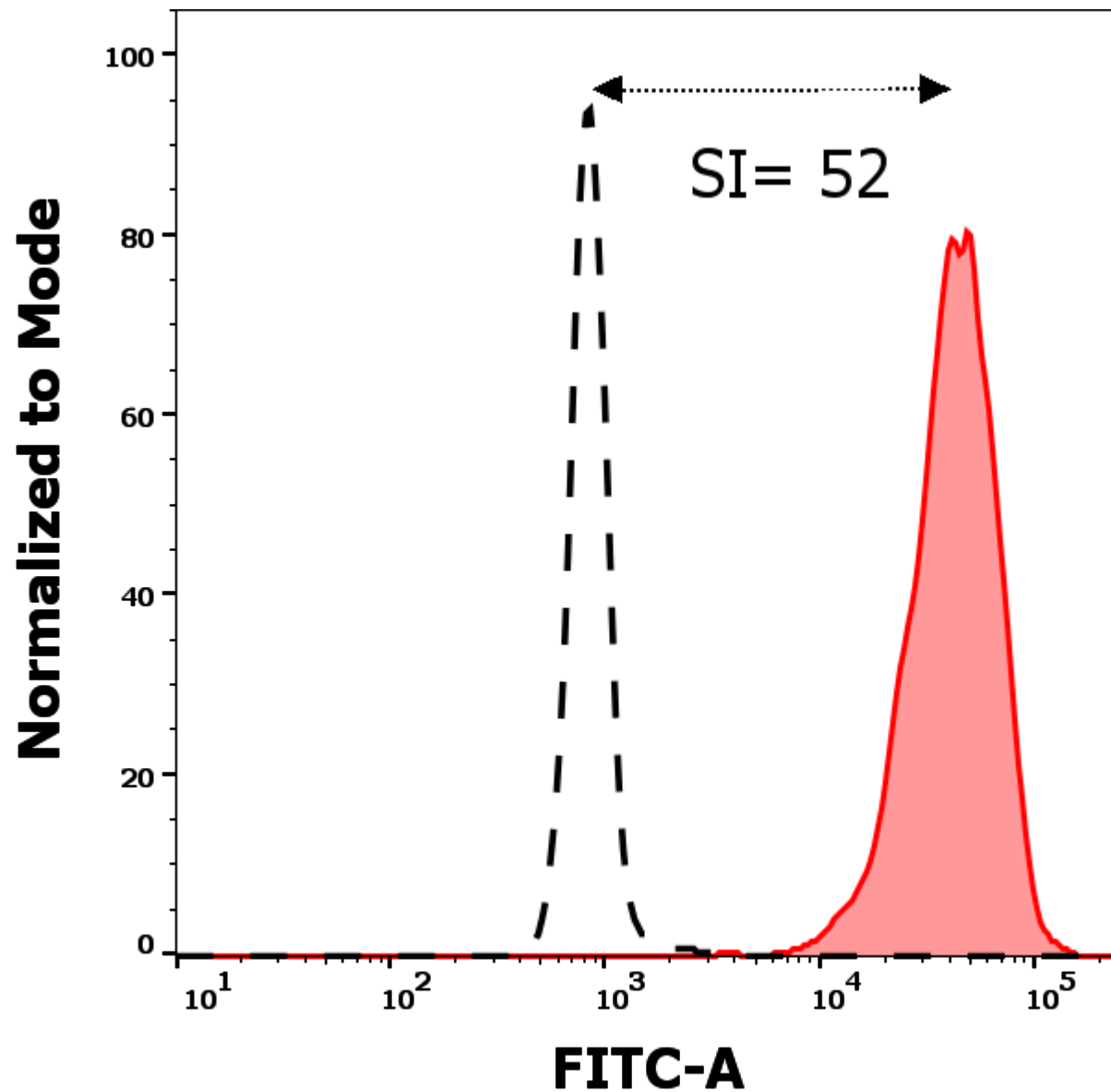
- **Kontrola**
- **Rozdělení na nestimulované a stimulované**
- **E.coli**



- **Kontrola**
- **Rozdělení na nestimulované a stimulované**
- **E.coli**
- **Počet (%) stimulovaných**



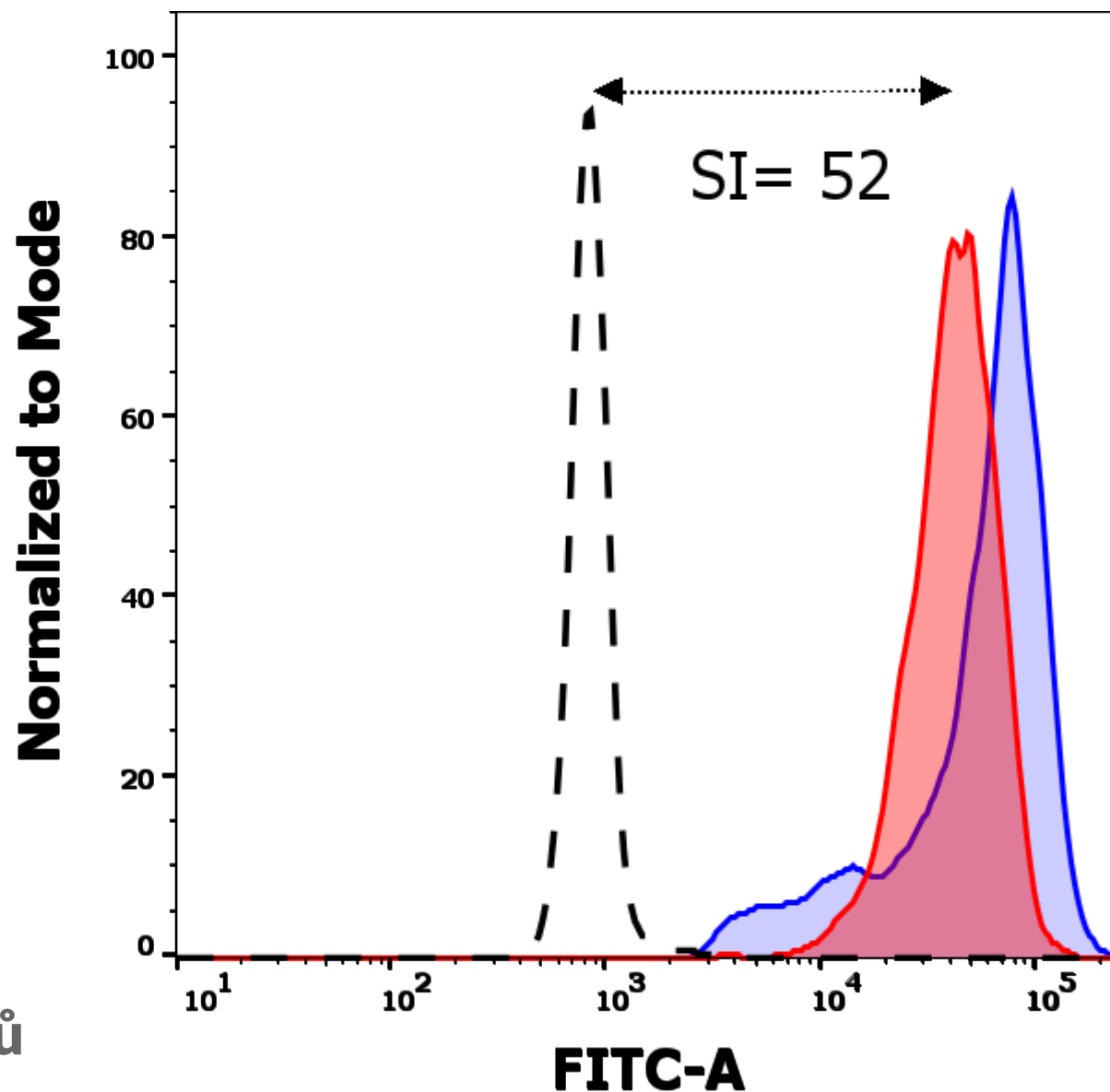
- **Kontrola**
- **Rozdělení na nestimulované a stimulované**
- **E.coli**
- **Počet (%) stimulovaných**
- **Poměr fluorescence stimulovaných a kontroly (SI)**



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- **Kontrola**
- **Rozdělení na nestimulované a stimulované**
- **E.coli**
- **Počet (%) stimulovaných**
- **Poměr fluorescence stimulovaných a kontroly (SI)**
- **Kontrola enzymů chemickou stimulací (PMA)**



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Zákaznické podněty - PMS

- Strmý pokles MFI a SI v čase po zpracování vzorku
- Lyophilizované reagenty mají po rozpuštění krátkou dobu použitelnosti
 - 1 den (v lednici)
 - 7 dní (-20 °C)
- Interpretace není jednoznačná (který parameter, % nebo SI, používat?)

FagoFlowEx Kit prototyp 2025

Plánování a analýza potřeb	Identifikace klinické potřeby Určení zamýšleného použití Analýza trhu, konkurence
Klasifikace prostředku (článek 47, příloha VIII, IVDR)	Rozdělení do tříd A, B, C, D podle rizikovosti
Vývoj a návrh prostředku	Návrh, vývoj a ověření designu
Analytická a klinická validace	Analytická validace - přesnost, opakovatelnost, citlivost, specifita Klinická hodnocení - sběr důkazů o klinické funkci
Posouzení shody	Pro třídy B, C, D zapojení notifikované osoby Prohlášení o shodě
Registrace a uvedení na trh	EUDAMED
Post-market surveillance a vigilance	Plán PMS a pravidelné aktualizace PMS zpráv, Periodic safety Update Report, Sledování incidentů a hlášení závazných incidentů

FagoFlowEx Kit prototyp 2025

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Post-market surveillance a vigilance	Plán PMS a pravidelné aktualizace PMS zpráv, Periodic safety Update Report, Sledování incidentů a hlášení závazných incidentů

Klinická potřeba

Fagocytóza (ingesce)

91551	STANOVENÍ FAGOCYTÁRNÍ AKTIVITY METODOU PRŮTOKOVÉ CYTOMETRIE
91449	STANOVENÍ FAGOCYTÁRNÍ AKTIVITY LEUKOCYTŮ INGESCÍ PARTIKULÍ (JEDEN SUBSTRÁT)



Oxidační vzplanutí (enzymy)

91553	STANOVENÍ OXYDATIVNÍHO VZPLANUTÍ GRANULOCYTŮ METODOU PRŮTOKOVÉ CYTOMETRIE
-------	--

Klinický účel použití

J Hum Immun. 2025;1(1). doi:10.70962/jhi.20250002

Disease names
are presented in
red

An asterisk is
added to
highlight
extremely rare
disorders

Syndrome associated		No Syndrome associated: DHR assay (or NBT test) ?	
		Normal	Abnormal
Cystic fibrosis. <i>CFTR</i> AR. # 219700 Pancreatic insufficiency, Respiratory infections, elevated sweat chloride	Leukocyte adhesion deficiency (LAD) <i>Recurrent bacterial infections. Impaired pus formation and wound healing. Skin ulcers. Leukocytosis with neutrophilia (WBC > 25000)</i>	Pulmonary alveolar proteinosis. <i>CSF2RA</i> *, XL # 300770. <i>CSF2RB</i> *, AR. # 614370 Alveolar proteinosis: severe respiratory distress	CGD: Early onset of severe and recurrent infections, abscesses. Autoinflammatory phenotype, Granulomata obstructing respiratory, urinary or gastrointestinal tracts. IBD and perianal disease: up to 30 % Pathogens: <i>S. aureus</i> , <i>Aspergillus</i> , <i>Candida</i> , <i>Burkholderia cepacia</i> , <i>Chromobacterium violaceum</i> , <i>Nocardia</i> , and invasive <i>Serratia marcescens</i> . BCG adverse effects in up to 20 %. Microscopic granulomas. XL CGD: <i>CYBB</i> (gp91 ^{phox}) # 306400 <i>NCF1</i> (p47 ^{phox}), AR # 233700 <i>CYBA</i> (p22 ^{phox}), AR # 233690 <i>NCF4</i> (p40 ^{phox}), AR # 613960 <i>NCF2</i> * (p67 ^{phox}), AR # 233710 <i>CYBC1</i> *, AR # 618935
Papillon-Lefèvre sd. <i>CTSC</i> AR. # 245000 Periodontitis, palmoplantar hyperkeratosis in some patients, premature teeth loss	LAD I. AR <i>ITGB2</i> # 116920 Delayed cord separation with omphalitis+++. Periodontitis leads to early loss of teeth. Severity of the disease correlates with the degree of deficiency in CD18 (FCM). (WBC 20,000–150,000 with 60–85 % neutrophils) LAD II (Congenital disorder of glycosylation, type IIc). AR <i>SLC35C1</i> # 266265 Mild LAD type 1 features with Hh-blood group, growth retardation, developmental delay, facial dysmorphism (depressed nasal bridge). LAD III AR <i>FERMT3</i> # 612840 Severe bleeding disorder. Defective platelet aggregation.	Pulmonary alveolar proteinosis. AutoAb to GM-CSF. Pulmonary alveolar proteinosis, cryptococcal meningitis, disseminated nocardiosis	
Localized juvenile periodontitis. <i>FPR1</i> AR. *136537 Periodontitis only		CCR2 deficiency*. <i>CCR2</i> , AR. # 219600 Pulmonary alveolar proteinosis (PAP), progressive polycystic lung disease, and recurrent infections, BCG disease.	
β-Actin. <i>ACTB</i> AD. #607371, # 620475 Mental retardation, short stature. Thrombocytopenia in some.	Rac 2 def*. <i>RAC2</i> AD LOF. # 608203 Poor wound healing. CGD-like phenotype (abnormal DHR assay)	WDR1 deficiency*. <i>WDR1</i> AR. # 150550 Poor wound healing, severe stomatitis, neutrophil nuclei herniate. Mild neutropenia. Some with autoimmunity.	G6PD def Class I. <i>G6PD</i> XL. # 300908 Hemolytic anemia, Infections.

Figure Legend:

Congenital defects of phagocyte number, function, or both. Functional defects of phagocytes.

AD: autosomal dominant inheritance; AR: autosomal recessive inheritance; BCG: Bacillus Calmette–Guerin; CD: cluster of differentiation; CGD: chronic granulomatous disease; FCM: flow cytometry; def: deficiency; DHR: dihydrorhodamine-1,2,3; GM-CSF: granulocyte/monocyte colony stimulation factor; IBD: inflammatory bowel disease; LAD: leukocyte adhesion deficiency; NBT: nitroblue tetrazolium; NK: natural killer cells; WBC: white blood cells; XL: X-linked inheritance. A searchable PDF file containing all figures in this article can be found under the “Supplements” tab.



From: The 2024 update of
IUIS phenotypic
classification of human
inborn errors of immunity

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Klinický účel použití

J Hum Immun. 2025;1(1). doi:10.

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XL CGD: **CYBB** (gp91^{phox}) # 306400

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Defekt oxidačního vzplanutí (CGD)

CYBB
CYTOCHROME b(558), BETA SUBUNIT
p91-PHOX
NADPH OXIDASE 2; NOX2
GP91-1
[Xp21.1-p11.4](#)

70%

RAS-RELATED C3 BOTULINUM
TOXIN SUBSTRATE 2; RAC2
[22q13.1](#)

NEUTROPHIL CYTOSOLIC FACTOR 2; NCF2
[1q25.3](#)

NEUTROPHIL CYTOSOLIC FACTOR 4; NCF4
Alternative titles; symbols
NCF, 40-KD
p40-PHOX
[22q12.3](#)

CYTOCHROME b(-254) CHAPERONE 1; CYBC1
[17q25.3](#)

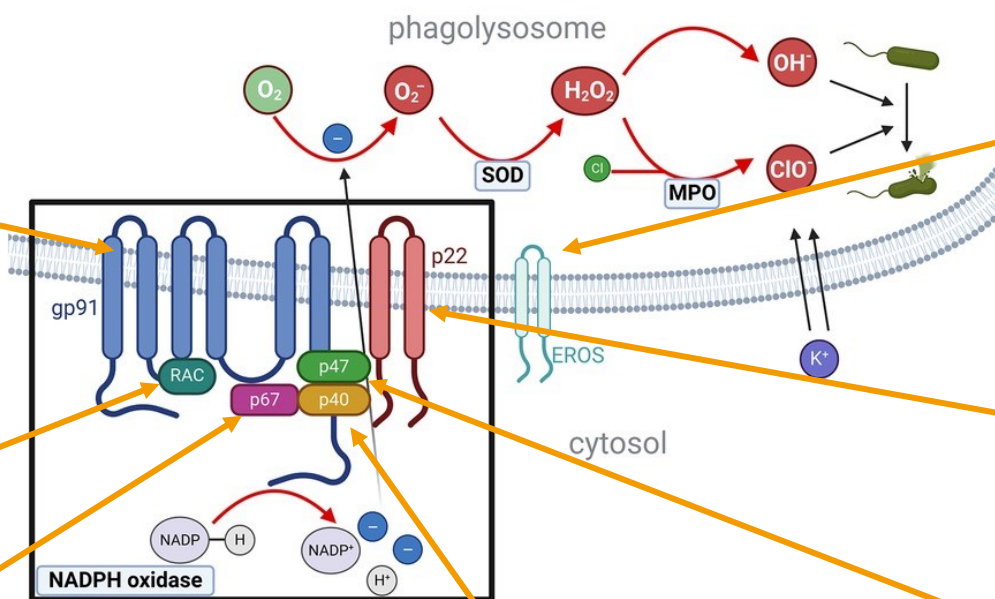
CYBA (CYTOCHROME b(558), ALPHA
SUBUNIT
p22-PHOX
CYTOCHROME b LIGHT CHAIN)
[16q24.2](#)

NEUTROPHIL CYTOSOLIC FACTOR 1; NCF1
[7q11.23](#)

25%

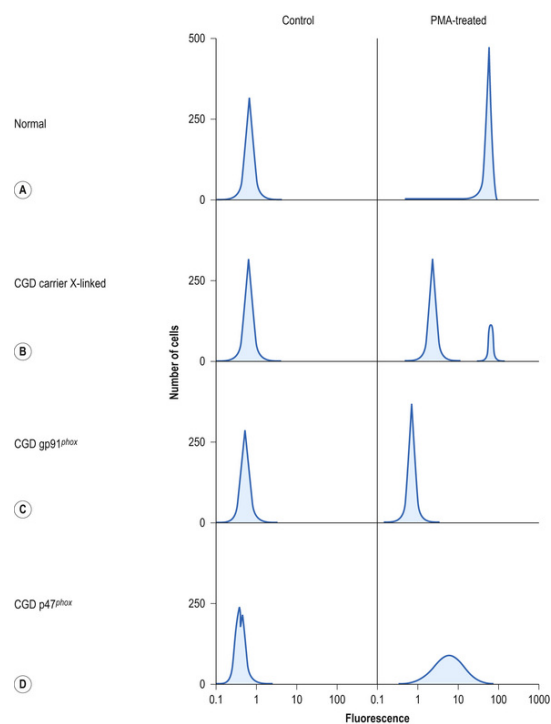
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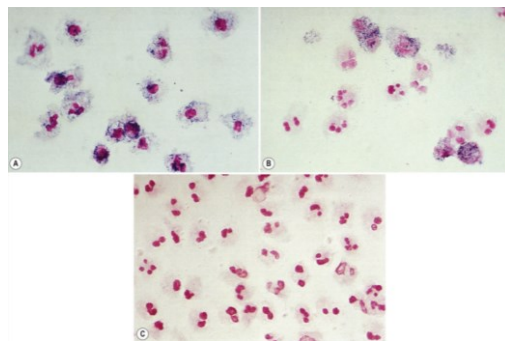


Testy oxidačního vzplanutí – konkurence

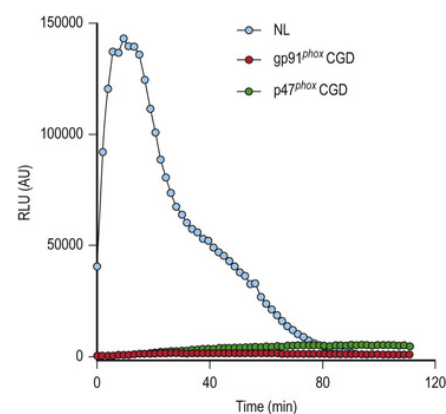
dhr assay



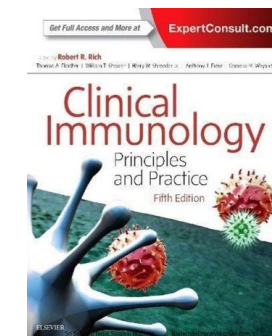
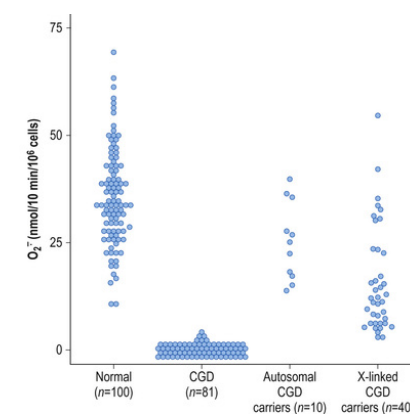
NBT



cytochrom c



luminol



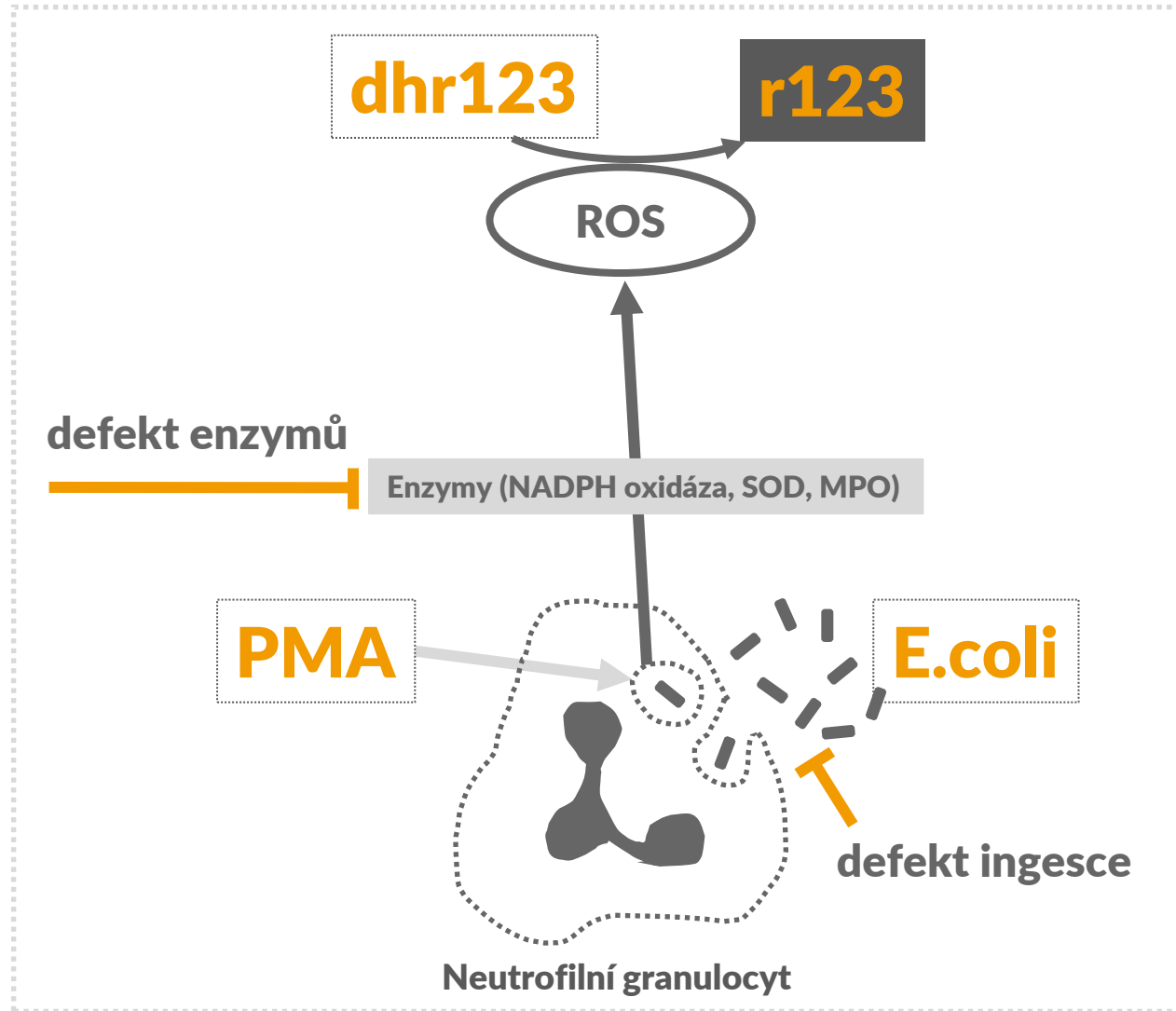
(2019)

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Klasifikace dle účelu použití

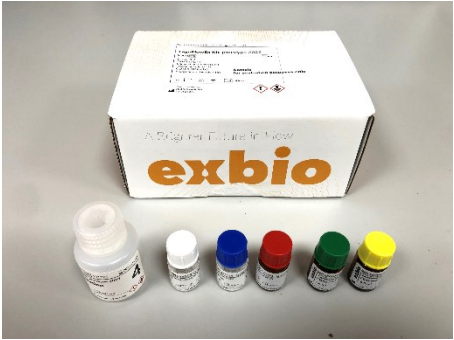
třída C



?

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FagoFlowEx Kit prototyp 2025



Složení

- E.Coli
- PMA
- DHR123
- Lysing solution
- **CD45**
- **Fluorescenční standard**

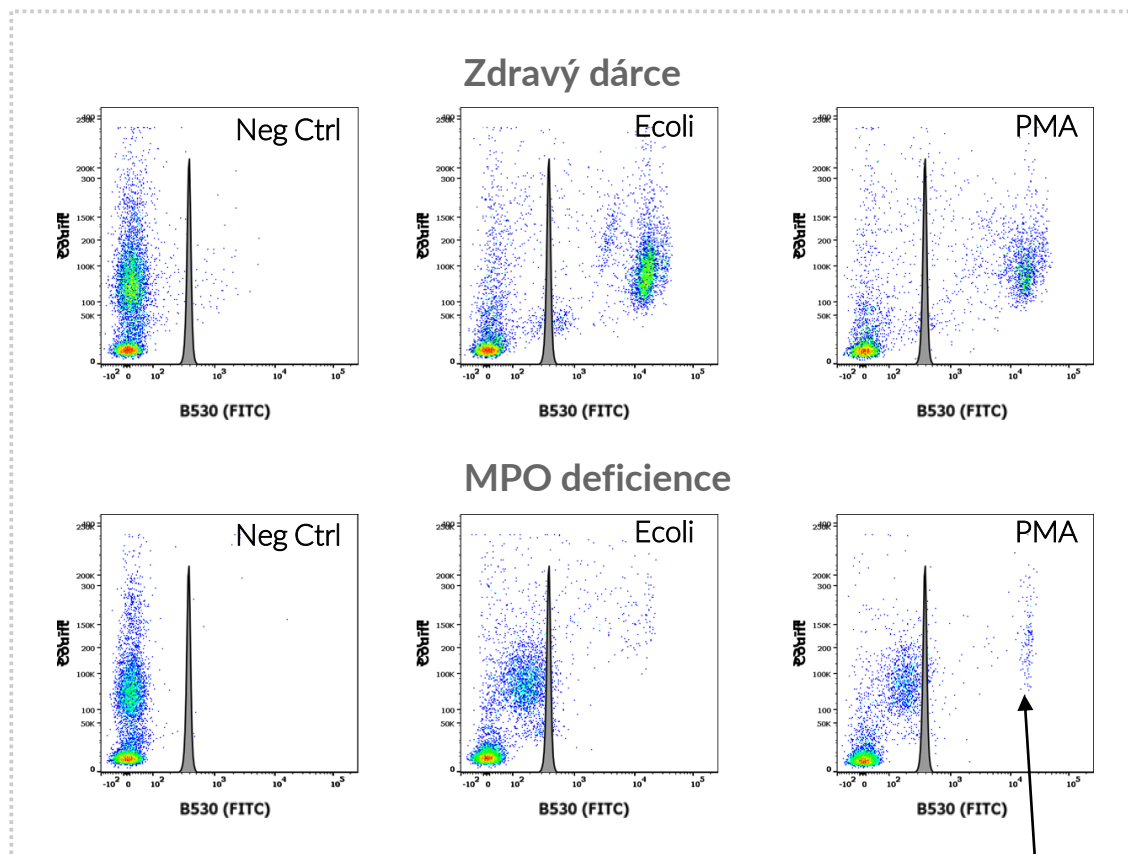
Vlastnosti

- **Isotonická „lýza“ erytrocytů**
- Stálý signál
- Skladování rekonstituovaných komponent v lednici
- Kvantifikace oxidačního vzplanutí ve vztahu k fluorescenčnímu standardu

Postup

1. Příprava zkumavek
2. DHR123
3. Stimulans
4. Vzorek krve
5. Inkubace 30 min/37stC
6. Lyzační roztok
7. **PBS**
8. CD45
9. Inkubace 20min
10. Měření

Oxidační vzplanutí prototyp 2025



Oxidační vzplanutí – vyhodnocení (poměr MFI/standard)

Zdravý dárce

PMA kontrola = 43

E.coli reakce = 40

MPO deficiency

PMA kontrola = 0.5

E.coli reakce = 0.4

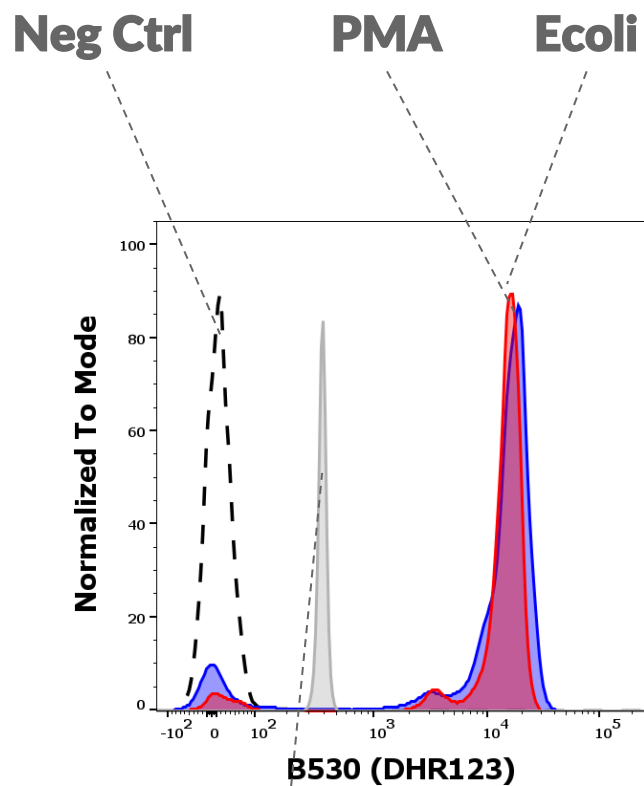
Oxidace DHR123
katalyzovaná
eosinofilní
peroxidázou u
MPOd vzorku

FagoFlowEx Kit prototyp 2025

exbio

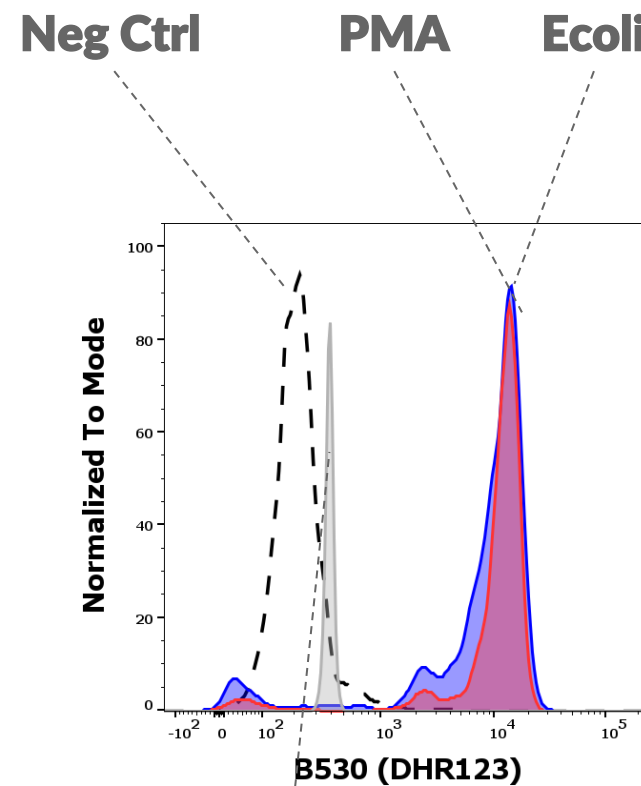
A Brighter Future in Flow

Čerstvě rozpuštěné



Fluorescenční kuličky 7th peak

4 týdny v lednici

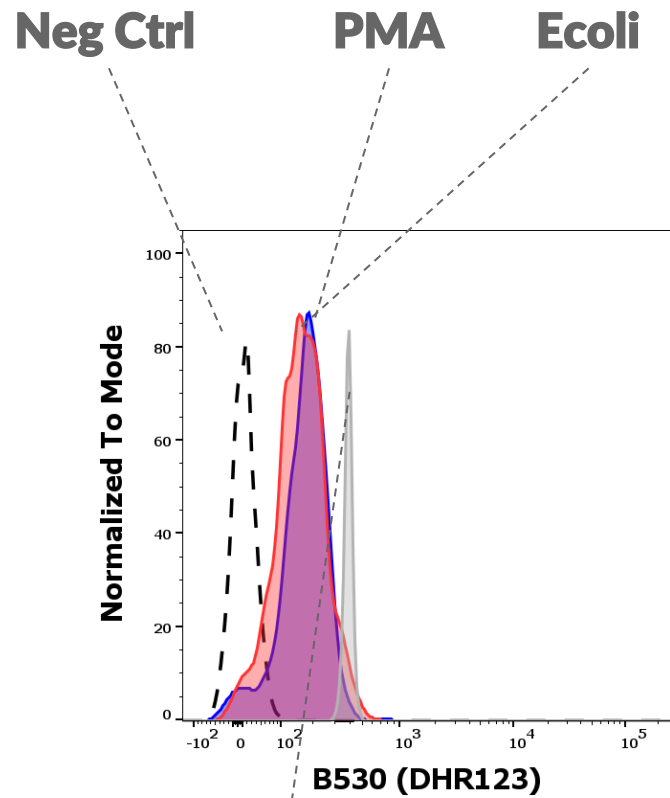


Fluorescenční kuličky 7th peak

FagoFlowEx Kit prototyp 2025

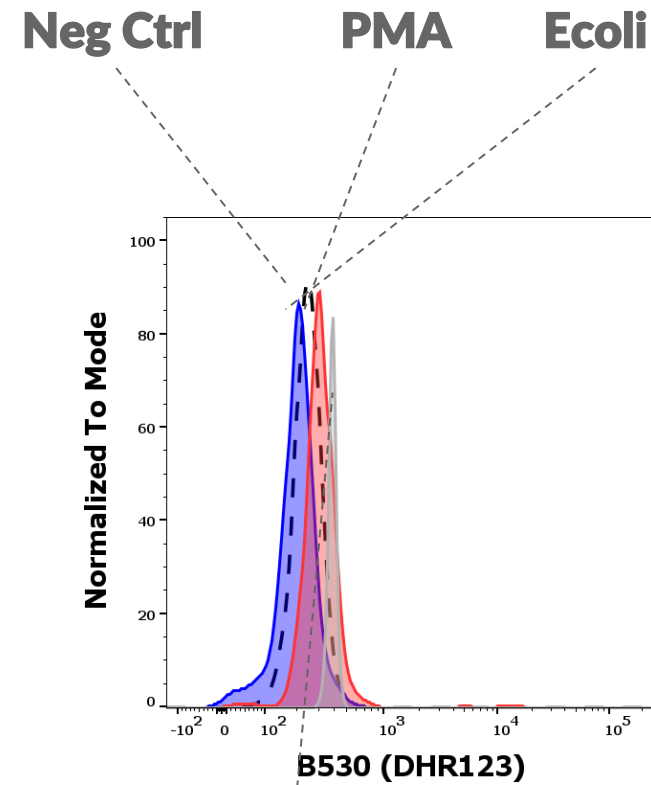
exbio
A Brighter Future in Flow

Čerstvě rozpuštěné



Fluorescenční kuličky 7th peak

4 týdny v lednici

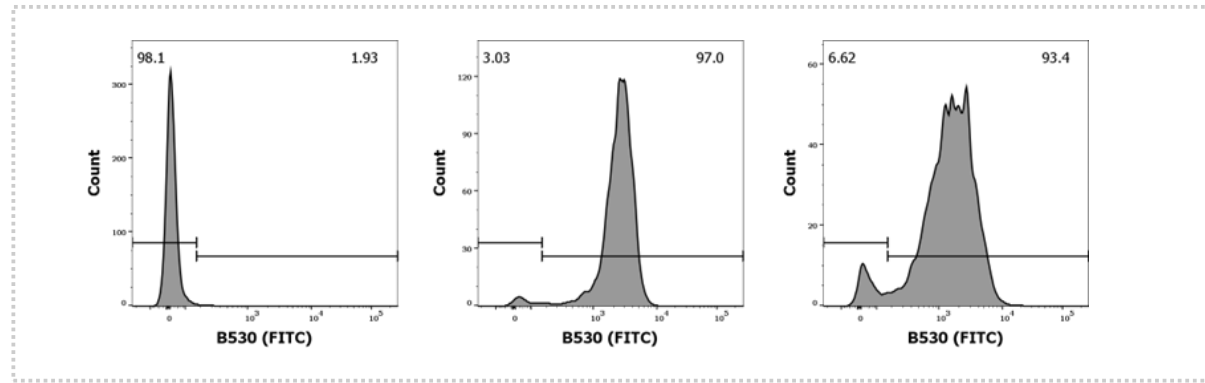


Fluorescenční kuličky 7th peak

FagoFlowEx Kit prototyp 2025

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A Brighter Future in Flow

Ingesce / fagocytóza prototyp 2025



Ingesce / Fagocytóza - vyhodnocení

Negativní kontrola = 1.9%

PMA kontrola = 97%

E.coli reakce = 93%

FagoFlowEx Kit prototyp 2025

Shrnutí

- FagoFlowEx kit bude inovován podle zákaznické zpětné vazby z PMS aktivit
- Souprava bude přizpůsobena diagnostice klinicky závažných imunodeficitů
- Vývoj ve fázi externího ověřování designu

