IngoFlowEx Kit

Cat.No. ED7040

Description

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.
Phagocytosis is a part of the innate defense mechanisms in which phagocytes ingest extracellular particulate material. The ability of phagocytosis is a characteristic feature of the so-called professional phagocytic cells (neutrophil and eosinophil granulocytes, monocytes and macrophages). The process includes particle binding, its ingestion and subsequent degradation. Plasma proteins (C3b and antibodies) assist the phagocytes in particle recognition and ingestion by covering the surface of foreign particles with readily recognizable ligands.
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 non-phagocyting 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.

Specification

E. coli FITC (Ready to use) contains fluorescein-labeled human plasma opsonized *E. coli* strain K−12 in suspension.
Quenching Solution (Ready to use) contains buffered solution of trypan blue.
Lysing Solution (10x concentrated solution) contains solution of fixation-lysing reagent.
Wash Buffer (25x concentrated solution) contains concentrated wash buffer.
DNA Staining Solution (Ready to use) contains buffered propidium iodide solution.

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.

Materials required but not provided

Deionized water (dH_2O), approx. 0.6 liter
Phosphate buffered saline (PBS), approx. 2 liters
5ml test tubes (12 × 75 mm)

Storage and handling

Store the IngoFlowEx Kit at 2-8 C. Expiration date is stated on reagent labels and on the IngoFlowEx Kit box.

Warnings and precautions

- Intended for research use only.
- Do not use reagents after their expiration date.
- Avoid contamination of reagents.
- Bacteria are provided as a ready-to-use suspension and their mean fluorescence intensity may change during storage, however the results interpreted as percent of phagocyting cells remains unaffected.
- Blood samples are considered as potentially infectious and must be handled with care. Use protective gloves and follow procedures for handling potentially infectious materials. Avoid contact of human blood samples with skin, eyes and mucous membranes.
- **Lysing Solution** (ED7040-3) contains formaldehyde, methanol, and diethylene glycol.
  H phrases
  H302+312+332: Harmful if swallowed, in contact with skin or if inhaled.
  H315: Causes skin irritation.
  H317: May cause an allergic skin reaction
  H319: Causes serious eye irritation.
  H335: May cause respiratory irritation.
  H351: Suspected of causing cancer
  H371: May cause damage to organs.
  H373: May cause damage to organs (kidney) through prolonged or repeated exposure if swallowed.
  P phrases
  P270: Do not eat, drink or smoke when using this product.
  P280: Wear protective gloves / protective clothing / eye protection / face protection.
  P301+P312: IF SWALLOWED: Call a POISON Center or doctor/physician if you feel unwell.
  P302+P352: IF ON SKIN: Wash with plenty of soap and water.
  P305+P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  P501: Dispose of contents/container to authorized facility for dangerous wastes.
- **Quenching Solution** (ED7040-2) contains 0.08% Trypan Blue.
  H phrases
  H350: May cause cancer.
  P phrases
  P201: Obtain special instructions before use.
  P270: Do not eat, drink or smoke when using this product.
  P280: Wear protective gloves / protective clothing / eye protection / face protection.
  P301+P312: IF SWALLOWED: Call a POISON Center or doctor/physician if you feel unwell.
  P302+P352: IF ON SKIN: Wash with plenty of soap and water.
  P308+P313: IF exposed or concerned: Get medical advice/ attention
  P501: Dispose of contents/container to authorized facility for dangerous wastes.
- See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product.
- **Blood must be collected into a tube containing heparin.** Anticoagulants EDTA and citrate negatively affects results of the analysis.
- Blood samples should be measured within 8 hours after collection.
- Reproducibility of the assay strongly depends on precise working (incubation times, temperature, pipetting).
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure the stable sensitivity of detectors.
- Flow cytometer may produce false results if the device has not been regularly calibrated and maintained appropriately.

Application

Measurement of phagocytic activity of human granulocytes and monocytes using flow cytometry.

Reagent preparation

Wash Buffer
Dilute Wash Buffer with PBS before use. Use the information given below as a quick guidance to dilution volumes.

No. of samples	Total (ml)	Wash Buffer (ml)	PBS (ml)
10	200	8	192
100	2000	80	1920

Assign with the date of preparation and store at 2-8 °C for maximum of 4 weeks.

Lysing Solution
Dilute Lysing Solution with deionized water. Use the information given below as a quick guidance to dilution volumes.

No. of samples	Total (ml)	Lysing Solution (ml)	dH_2O (ml)
10	40	4	36
100	400	40	360

Assign with the date of preparation and store at 2-25 °C for maximum of 4 weeks.

E. coli FITC
Before use thoroughly mix the content of the vial using vortex mixer.

Required for handling

Cylinders and beakers to dilute the reagents
Vortex mixer
Ice bath (ice cubes or crushed ice)
Incubation racks for the test tubes
Automatic pipettes with disposable tips
Thermal incubator or water bath (37 °C)
Centrifuge with rotor for 5ml tubes
Flow cytometer - blue laser excitation 488 nm, light emission at 525 nm (FITC channel) and 617 nm (PE channel).

Procedure

Allow **diluted Wash Buffer** to cool to 2-8 °C.
Allow **diluted Lysing Solution** to reach room temperature.
Place **E. coli FITC**, **Quenching Solution** and **DNA Staining Solution** on ice.
Place an incubation rack for test tubes in the ice bath.
For each blood sample prepare two test tubes:
- the reaction with *E. coli*
- the control reaction (without *E. coli*)

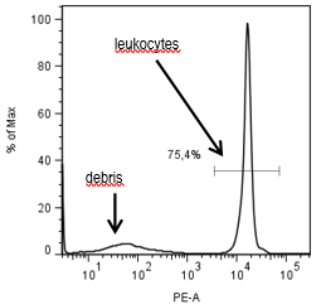
1. Pipette **50 µl of blood** into the test tubes.
2. Place the tubes on ice for 10 minutes.
3. Add **10 µl of E. coli FITC** to the tube intended to perfom reaction with *E. coli* and mix using a vortex mixer. Return the tube to ice.
4. Do not add anything into the **control** tubes.
5. Transfer the rack with tubes into a thermal incubator set to 37 °C (or a water bath set to 37 °C).
6. Incubate for 30 minutes (10 minutes if using the water bath).
7. Return the rack with tubes to ice and incubate for 5 minutes.
8. Add **100 µl of cold Quenching Solution** (2−8 °C) and mix well. Keep the tubes on ice.
9. Add **3 ml of cold diluted Wash Buffer** (2−8 °C) and centrifuge the tubes at 200 g for 5 min.
10. Remove the supernatant by aspiration into a container with an appropriate disinfectant. Do not disturb the pellet.
11. Repeat the wash (steps 9 and 10).
12. Resuspend the pellet in the residual volume of diluted Wash buffer using vortex mixer.
13. Add **2 ml of diluted Lysing Solution** (room temperature), mix and incubate for 20 minutes at room temperature in the dark.
14. Centrifuge the cells at 200 g for 5 min.
15. Remove the supernatant but do not disturb the pellet.
16. Add **3 ml of cold diluted Wash buffer** and centrifuge at 200 g for 5 min.
17. Remove supernatant and resuspend the pellet in **300 µl of cold DNA Staining Solution** (2-8 °C).
18. Incubate for 10 minutes on ice in the dark.
19. Measure with flow cytometer **within 2 hours**.

Flow Cytometric Analysis

Setting up the cytometer before the first run
The color compensation for spectral overlap may be assessed with performing a set of single reagent stained blood samples. To perform the single stained reactions, follow the described procedure but use only one fluorescent component at a time.
a) Blood sample with Ecoli FITC (omit Quenching Solution and DNA Staining Solution)
b) Blood sample with Quenching Solution (omit E. coli FITC and DNA Staining Solution)
c) Blood sample with DNA Staining Solution (omit E. coli-FITC and Quenching Solution).
Use these measurements to adjust the cytometer settings.

Analysis of samples
Set a gate in PE channel (Figure 1) and acquire the sufficient number of cellular events (at least 10,000 PE-bright events). This gate excludes debris and clumps of bacteria from further analysis.

Fig. 1 Gate for cells that exclude debris and bacterial clumps from further analysis.



Visualize the "PE bright" gated events in the side-scatter (SSC) versus forward-scatter (FSC) dot-plot and set gates around granulocytes and monocytes (Figure 2).
Then bring the gated subpopulations to histograms in FITC channel (Figures 3a and 3b). Use the control reaction histogram to place the discrimination line. A single discrimination line setting is usually applicable for all tested blood samples within the run.

Fig. 2 Gates for granulocytes and monocytes.

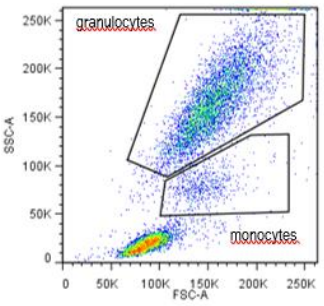


Fig. 3a Overlay of histograms of granulocyte subpopulation.

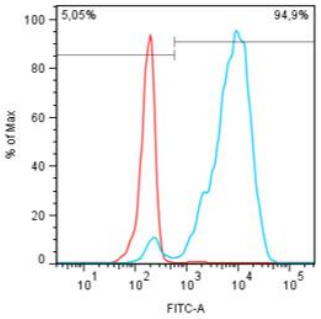
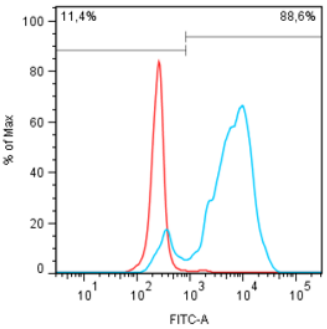


Fig. 3b Overalay of histograms of monocyte subpopulation.



The results can be interpreted as
1) percent of phagocytes containing fluorescent *E. coli* or
2) mean fluorescence of phagocyting cells.
The percent of actively phagotycing cells is elswere called the **phagocytic activity**. The following table shows the average values of actively phagocytic cells that were calculated from data obtained by measurement of blood samples from healthy donors.

Subpopulation of cells	% active phagocytes (average +/- 2SD)
Granulocytes	91 (83-100)
Monocytes	82 (72-94)

Repeatability

Repeatability was determined by measuring four parallels from different healthy donors in different days and the variability is expressed as the average coefficient of variation.

	% active cells (range)	average CV (%)
Granulocytes	83-97	1.8
Monocytes	73-91	4.8

References

M. O'Gorman. Clinical evaluation of Myeloid and Monocytic Cell functions in Manual of Molecular and Clinical Laboratory Immunology edited by B. Detrick, 7th edition, AMS Press 2006, Page 272-280

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Trademarks

n/a

Revision History

• Version 1, ED7040_TDS_v1
Initial Release
• Version 2, ED7040_TDS_v2
Diluent change for Wash Buffer, instead of diluting Wash Buffer with deionized water (dH_2O) it is recommended to dilute Wash Buffer with Phosphate buffered saline (PBS).
A text "The mixture is classified as dangerous according to the criteria of directive(s) 67/548/EEC and/or 1999/45/EC" was deleted from Precautions.
• Version 3, ED7040_TDS_v3
R- and S-phrases and hazard symbol were changed to H- and P-phrases and GHS symbols according to Regulation (EC) No 1272/2008.
• Version 4, ED7040_TDS_v4
Format corrections.
• Version 5, ED7040_TDS_v5
The company logo changed. IFU layout changed. The "Consult instructions for use", "Keep away from sunlight" and "For Research use only" symbols were added to Symbols. Manufacturer postal code changed from 25242 to 25250.
• Version 6, ED7040_TDS_v6
Product Use Limitation text was refined.

Symbols

REF	Catalog number
LOT	Batch code
	Use-by date
	Temperature limits
	Consult instructions for use
	Keep away from sunlight
	Manufacturer
RUO	For Research use only. Not for use in diagnostic or therapeutic procedures.

exbio

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100 tests | Cat.No. **ED7040**

For Research use only.
Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet

Version ED7040_TDS_v6_EN
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