IngoFlowEx Kit

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

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7040_TDS_v8_EN

Date of Issue: 10-02-2026

Symbols used in the product labeling

RUO	Research Use Only		Keep Dry Keep away from rain
	Manufacturer		Caution
	Consult instructions for use	CONC 10×	Concentrated solution (10x)
	Contains sufficient for <n> tests	CONC 25×	Concentrated solution (25x)
REF	Catalogue number	CONTENTS	Contents
LOT	Batch code		
	Use by date		
	Temperature limit		
	Keep away from sunlight		

Description

The product is For Research Use Only. Diagnostic or therapeutic applications are strictly forbidden.

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. A negative control without E. coli is used to set the discrimination boundary between phagocytosing and non-phagocytosing cells. Non-ingested bacteria are removed through repeated washes. Any remaining extracellular or surface-bound bacteria are quenched using trypan blue, a vital dye that does not penetrate the cell membrane. Erythrocytes are lysed, cells are fixed, and DNA is stained with propidium iodide to differentiate nucleated cells from debris or clumps of bacteria. The *E. coli* bacteria used in the test were opsonized with human AB plasma, ensuring that the results are not primarily dependent on the opsonizing activity of the tested blood sample.

Reagent(s) provided

Contents

The product IngoFlowEx Kit is sufficient for 100 tests and is provided with the following reagents:

E. coli FITC ED7040-1 (1 vial) containing 1 ml of fluorescein-labeled *E. coli* strain K−12 opsonized with human plasma.

Quenching Solution ED7040-2 (1 bottle) containing 20 ml of 0.2% trypan blue solution.

Lysing Solution ED7040-3 (1 bottle) containing 60 ml of a 10X concentrated lysing solution with fixative.

Wash Buffer ED7040-4 (1 bottle) containing 80 ml of a 25X concentrated solution.

DNA Staining Solution ED7040-5 (1 bottle) containing 60 ml of propidium iodide solution.

Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

Phosphate buffered saline (1× PBS), pH 7.2 – 7.4

Equipment required

Automatic pipette with disposable tips (10 µl – 5 ml) for pipetting specimen and reagents

Thermal incubator or water bath (37 °C)

Ice bath (ice cubes or crushed ice)

Cylinders and beakers to dilute the reagents

Racks for the test tubes

Vortex mixer

Centrifuge

Flow cytometer with laser excitation source (488 nm), detectors for scattered light, optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 2.

Table 2 Spectral characteristic of fluorochromes use in the product

Flurochrome	Excitation [nm]	Emission [nm]
FITC	488	525
Propidium iodide	488	636

Storage and handling

Store at 2-8 °C.

Avoid prolonged exposure to light.

See Section Procedure (Preparation of reagent(s) provided) for information about the storage conditions and stability of working solutions (where applicable).

Warnings, precautions and limitations of use

GHS Hazard Classification

WARNING: Quenching Solution (ED7040-2) contains trypan blue (CAS No. 72-57-1) in concentrations classified as hazardous.

Label elements	Signal word
	**Danger**
H-phrases	H350: May cause cancer.
P-phrases	P201: Obtain special instructions before use. P280: Wear protective gloves. P308+P313: IF exposed or concerned: Get medical advice/ attention. P405: Store locked up. P501: Dispose of contents/container to by handing over to the person authorized to dispose of waste or by returning to the supplier.

WARNING: Lysing Solution (ED7040-3) contains formaldehyde (CAS No. 50-00-0), methanol (CAS No. 67-56-1) and 2,2'-oxybisethanol (CAS No. 111-46-6) in concentrations classified as hazardous.

Label elements	Signal word
 	**Danger**
H-phrases	H302: Harmful if swallowed. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. H331: Toxic if inhaled. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. H371: May cause damage to organs. H373: May cause damage to the kidneys through prolonged or

	repeated exposure if swallowed. EUH071: Corrosive to the respiratory tract.
P-phrases	P201: Obtain special instructions before use. P260: Do not breathe vapours. P280: Wear protective gloves/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P403+P233: Store in a well-ventilated place. Keep container tightly closed.

Consult Safety Data Sheet (SDS) available on the product page at www.exbio.cz for the full information on the risks posed by chemical substances and mixtures contained in the Product and how they should be handled and disposed.

Biological Hazard

Human biological samples and blood specimens and any materials coming into contact with them are always considered as infectious materials.

Use personal protective and safety equipment to avoid contact with skin, eyes and mucous membranes.

Follow all applicable laws, regulations and procedures for handling and disposing of infectious materials.

Evidence of deterioration

Normal appearance of the Lysing Solution is a clear liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of the Wash Buffer is a clear liquid. If you encounter signs of precipitation, bring the solution to the room temperature and let the precipitate to dissolve.

Normal appearance of the Quenching Solution is a dark blue liquid. If you encounter signs of precipitation bring the solution to the room temperature and let the precipitate to dissolve.

Normal appearance of the E. coli FITC is an opalesque light yellow liquid with a resuspendable sediment.

Limitation of use

Do not use after the expiry date stated on the product labels.

Specimen

Use peripheral blood collected in specimen receptacle classified as a medical product with heparin anticoagulant. Anticoagulants EDTA and citrate negatively affects results of the analysis.

Blood samples should be measured within 8 hours after collection. Blood

specimen in the collection tube must be stored at room temperature (20-25 °C). Do not refrigerate.

Procedure

Preparation of reagent(s) provided

Wash Buffer

Dilute Wash Buffer with PBS and store at 2-8 °C. A minimum of 9 ml is needed per a test tube (18 ml per a specimen test). Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container. The diluted Wash buffer (1X) is stable for 1 month when stored in a liquid dispenser or closed container at 2-8 °C.

Use the following table as a quick guidance to the required volume.

No. of test tubes	Total (ml)	Wash Buffer (ml)	PBS (ml)
10	200	8	192

Lysing Solution

Dilute Lysing Solution with deionized water and store at 2-8 °C or room temperature. A minimum of 2 ml is needed per a test tube (4 ml per a specimen test). Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container. The diluted Lysing solution (1X) is stable for 1 month when stored in a liquid dispenser or closed container at 2-8 °C or at room temperature.

Use the following table as a quick guidance to the required volume.

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

E. coli FITC

Place the reagent into the ice bath or keep the reagent in a refrigerator.

Quenching Solution

Place the reagent into the ice bath or keep the reagent in a refrigerator.

DNA Staining Solution

Place the reagent into the ice bath or keep the reagent in a refrigerator.

Preparation of other equipment

Prepare a rack for the round bottom test tubes (12 × 75 mm) and place it in an ice bath.

Sample stimulation protocol

1. For each specimen, prepare two round bottom test tubes (12 × 75 mm).

   Label them according to the specimen ID and according to the stimulation:

   - E. coli - a sample incubated with fluorescent bacteria,
   - Negative control - a sample incubated without *E. coli* bacteria.

2. Pipette 50 µl of well-mixed blood specimen into the bottom of the test tubes.

   Cool the samples in tubes for 10 minutes in the ice bath.

3. Add 10 µl of well-mixed E. coli FITC to the E. coli labelled tube.

   Vortex to mix and return the tube into the ice bath.

   Do not add anything into the Negative control tube.

4. Transfer the rack with tubes into 37 °C and incubate:

   - for 30 minutes in a thermal incubator or
   - for 10 minutes in a water bath.

5. Return the tubes on ice for 5 minutes.

Sample staining protocol

1. Pipette 100 µl of cold Quenching Solution to the bottom of the test tubes.

   Vortex to mix the content.

2. Wash:

   Add 3 ml of cold diluted (1X) Wash Buffer.

   Centrifuge for 5 min at 200× g, discard the supernatant into a container with an appropriate disinfectant.

   NOTICE: Centrifugation at 2-8 °C makes the cellular pellet too firm, making erythrocytes partially resistant to lysis. The procedure was designed for centrifuges without cooling capabilities.

3. Repeat the wash:

   Add 3 ml of cold diluted (1X) Wash Buffer.

   Centrifuge for 5 min at 200× g, discard the supernatant into a container with

an appropriate disinfectant. Resuspend the cell pellet in the residual volume using a vortex mixer.

4. Add 2 ml of diluted (1X) Lysing Solution (room temperature) and, if needed, pipette up and down to resuspend any remaining aggregates.

   Incubate for 20 minutes at room temperature in the dark.

5. Centrifuge for 5 min at 200× g, discard supernatant.

6. Final wash:

   Add 3 ml of cold diluted (1X) Wash Buffer.

   Centrifuge for 5 min at 200× g, discard supernatant.

7. Resuspend the cell pellet in 300 µl of cold DNA Staining Solution and incubate on ice in the dark for 10 minutes.

8. Acquire the stained sample immediately using flow cytometer. If the stained sample will not be acquired immediatelly, store at 2-8 °C in the dark and analyze within 2 hours.

Flow cytometry analysis

The flow cytometer selected for use with the product IngoFlowEx Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturers instructions.

If not maintained properly the flow cytometer may produce false results.

Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section Equipment required.

Set voltages on the fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interfere with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.

Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

For measured data analysis it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

To analyze a sufficient number of phagocytic cells (~3,000 neutrophils), acquire at least 10,000 leukocytes (propidium iodide stained events) per sample.

Determination of the fluorescence spillover and adjusting the cytometer compensation

Under a standard cytometer settings the spectral overlap is not a serious concern, but if needed the spillover between the detectors used in the IngoFlowEx Kit measurements can be determined by performing a set of single component stained reactions with a sample from a healthy blood donor.

1. A blood sample incubated with E. coli FITC and processed according to the protocol but without adding the Quenching Solution and DNA Staining Solution.
2. A blood sample incubated without E. coli FITC and processed according to the protocol (with Quenching Solution), but without adding the DNA Staining Solution.
3. A blood sample incubated without E. coli FITC and processed according to the protocol (with DNA Staining Solution), but without adding the Quenching Solution.

Record fluorescence intensities for each channel and adjust compensation settings based on the overlap values calculated from the measurements.

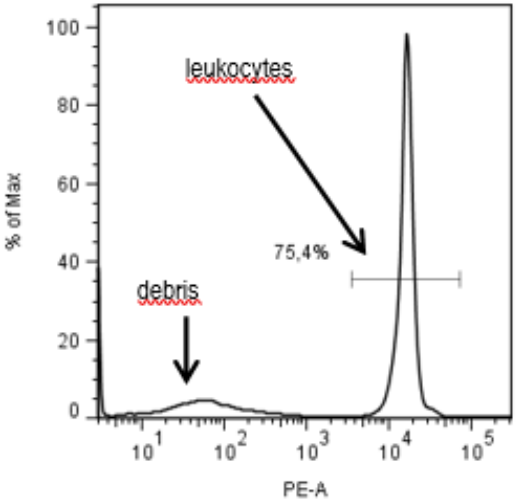
Data analysis

Visualize data as a histogram where X-axis represents fluorescence intensity PE channel.

> **NOTICE:** To analyze a sufficient number of phagocytic cells (~3,000 neutrophils), acquire at least 10,000 leukocytes PE bright, i.e. propidium iodide stained events, per sample.

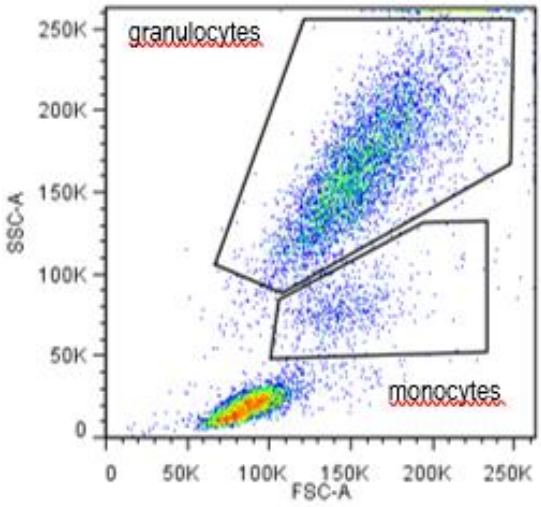
Create gate that excludes dim events (debris and clumps of bacteria) from further analysis (Figure 1).

Figure 1 Gating leukocytes (PE bright events) to exclude debris and bacterial clumps from further analysis.



Visualize leukocytes in the side-scatter (SSC) versus forward-scatter (FSC) plot. Set gates for granulocytes and monocytes (Figure 2).

Figure 2 Delineation of granulocytes and monocytes.
(data acquired on BD FACSCanto™)



Visualize the granulocytes and monocytes as histograms where X-axis represents fluorescence intensity in FITC channel. Use the negative control to set the discrimination gate between non-phagocyting (FITC negative) and phagocyting (FITC positive) cells.

Figure 3a Overlay of granulocytes as FITC channel fluorescence intensity histogram from negative control (red line) and from the E. coli FITC reaction (blue line).

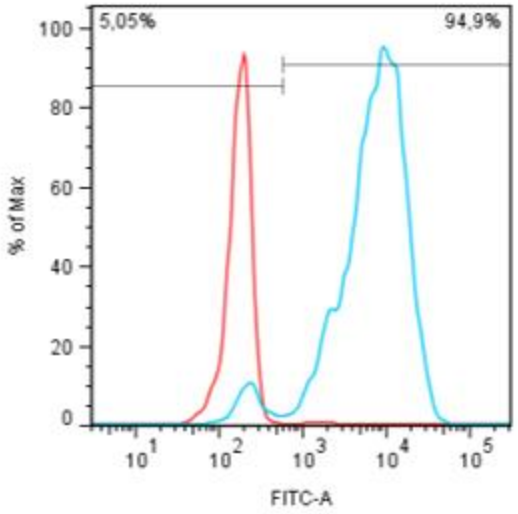
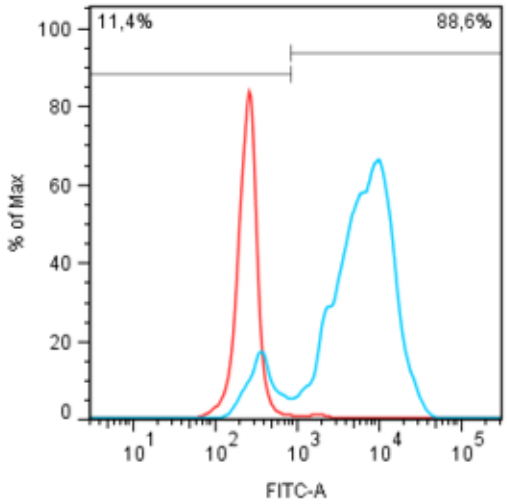


Figure 3b Overlay of monocytes as FITC channel fluorescence intensity histogram from negative control (red line) and from the E. coli FITC reaction (blue line).



Interpretation of data

1) Percent of phagocyting cells (containing fluorescent *E. coli* bacteria)

or

2) Quantity of ingested bacteria by comparing the mean fluorescence of phagocyting cells between specimens.

NOTICE: Bacteria are provided in suspension and the mean fluorescence intensity of bacteria decreases during storage. The comparisons between the quantity of ingested bacteria can only be done if the samples were tested at the same day.

Expected values

The average values of actively phagocytic cells were determined by examination of samples from thirteen healthy blood donors.

Leukocyte subset	Percent of phagocyting cells Average (+/- 2SD)
Granulocytes	91 (83-100)
Monocytes	82 (72-94)

Repeatability

The repeatability of the assay was measured on thirteen blood samples in quadruplicates. Coefficients of variation (CV) are provided in the table below.

	Measured range (percent of phagocyting cells)	CV (%) (min-max)
Granulocytes	83-97	1.8 (0.1-4.9)
Monocytes	73-91	4.8 (1.0-15.8)

References

1) 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.

Use of Third Party Trademarks

FlowJo™, FACSCanto™ are registered trademarks of Becton, Dickinson and Company, VenturiOne® is a registered trademark of Applied Cytometry, Infinicyt™ is a registered trademark of Cytognos S.L..

Revision History

Version 8, ED7040_TDS_v8

Change in hazard classification for component ED7040-3 Lysing Solution.

Manufacturer

EXBIO Praha, a.s.

Nad Safinou II 341

25250 Vestec

Czech Republic

Contact Information

info@exbio.cz

technical@exbio.cz

orders@exbio.cz

www.exbio.cz

NOTICE: Any serious incident that has occured in relation to the product shall be reported to the manufacturer and the local competent authority.