



Antibodies

Cell Proliferation and Apoptosis

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Proliferation

pp. 8–11

Apoptosis

FlowEx®



EXBIO Praha

Dedicated to Superior Cytometry Reagents

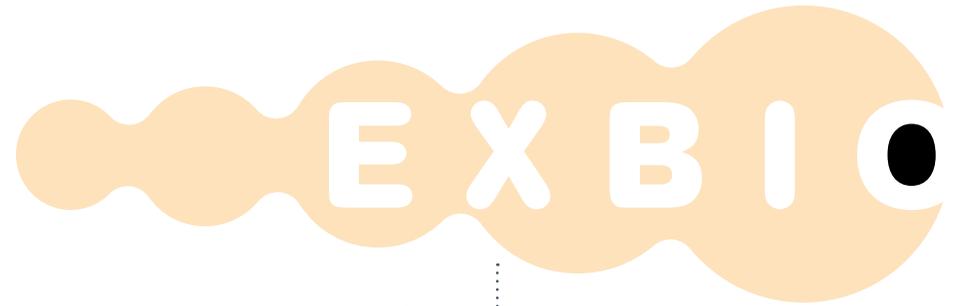
Mission

EXBIO Praha strives to exceed the most demanding customer expectations in the field of analytical cytometry by providing a comprehensive range of high quality products and services at affordable prices.

EXBIO Praha is a dynamic privately-owned European biotechnology company focused on design, development, manufacture and sale of monoclonal antibodies and other reagents for research and clinical applications.

Who we are

EXBIO Praha was established at the beginning of 1999 and soon became a trusted and reliable OEM supplier to all leading antibody vendors. Our flow cytometry antibodies have become widely recognized reagents among life science researchers and diagnostic laboratories. In addition to OEM supplies, we distribute our products worldwide under the EXBIO trademark through a network of experienced local distributors.



Our portfolio

Flow cytometry validated antibodies for a broad range of research applications (immunology, hematology, cancer research, etc.) available in many colors and formulations (suitable for 8-color immunophenotyping).

Pre-mixed antibody conjugate cocktails for *in vitro* diagnostic (CE IVD) applications (immunophenotyping of white blood cells).

Flow cytometry assays (FlowEx® Kits) for comprehensive diagnosis and analysis of patient health status: **FagoFlowEx® Kit** for examination of **phagocytosis (CE IVD)**, **BasoFlowEx® Kit** for analysis of **allergy specificity (CE IVD)**, **ApoFlowEx® FITC Kit** for detection of **apoptosis (RUO)** and **SpermFlow Kit** for evaluation of human **semen quality (RUO)**.

Other monoclonal antibodies for applications such as Western blotting, immunohistochemistry, immunoprecipitation, ELISA or functional *in vivo* studies. Antibodies to cytokeratins, betaIII-tubulin, sHLA-G and IgE belong to our best sellers.

sHLA-G ELISA kit for determination of soluble forms of Human Leukocyte Antigen-G (sHLA-G) in amniotic fluid, cell culture supernatant, plasma and serum.



Antibodies

Monoclonal and polyclonal antibodies and kits for research applications.



Diagnostics

Reagents and kits for flow cytometry-based *in vitro* diagnostic applications:

- Immunophenotyping
- Examination of allergy
- Examination of phagocytosis

BrdUFlowEx[®] FITC Kit

Catalog No.: **ED7073**
 Regulatory status: **RUO**
 Quantity: **100 tests**

Reagents provided: **Fix and Lysing Solution**
Permeabilizing Solution
Atomic Scissors Solution 1
Atomic Scissors Solution 2
Atomic Scissors Solution 3
Anti-BrdU FITC
7-AAD
BrdU

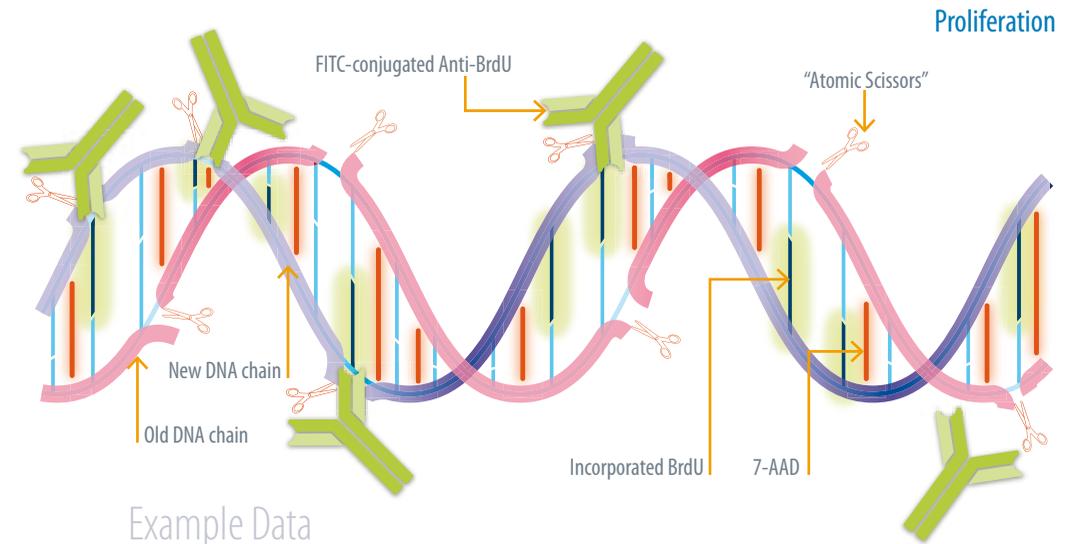
The **BrdUFlowEx[®] FITC Kit** is intended for labeling and subsequent detection of proliferating cells by flow cytometry. The method is based on incorporation of BrdU (5-bromo-2-deoxyuridine, a thymidine analog) into the DNA of proliferating cells, and its detection using fluorescently labeled anti-BrdU antibody. Total DNA is detected using 7-AAD (7-amino-actinomycin D). Long time exposures to BrdU (several hours to overnight) are used for experiments with identification of cycling versus non-cycling populations, whereas short time exposures allow analysis of cell cycle kinetics. To reveal the incorporated BrdU, the DNA is exposed to so called "atomic scissors", when deoxyribose moiety of DNA strand is attacked by copper ions in presence of oxygen. This approach mediates mild DNA damage and allows simultaneous detection of other cellular nuclear proteins including those which are usually

lost during other BrdU revealing methods (e.g. PCNA-1). The reaction requires considerable oxygen supply, which is achieved by shaking the reaction mixture. Following this unmasking step the cells are incubated with fluorescently labeled BrdU-specific antibody. The DNA is simultaneously labeled by 7-AAD, which allows the user to analyze also particular cell cycle phase (G0/1, S, G2/M).

The **BrdUFlowEx[®] FITC Kit** is compatible with EdU staining, as the anti-BrdU antibody included in this kit (clone MoBu-1) does not crossreact with EdU. (Reagents for EdU staining are not provided in the kit.)

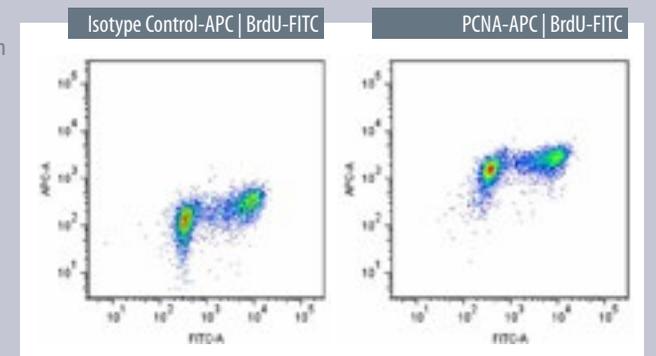
This product was developed with the support of the Technology Agency of the Czech Republic (Project TA03010719).

BrdUFlowEx[®] FITC Kit



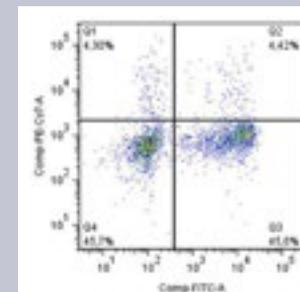
Example Data

The BrdUFlowEx[®] FITC Kit can be used for simultaneous detection of DNA synthetic activity and nuclear proteins. The example shows analysis of incorporated BrdU (anti-BrdU FITC) and PCNA (anti-PCNA APC) in Jurkat cells pulsed with 10 μ M BrdU.

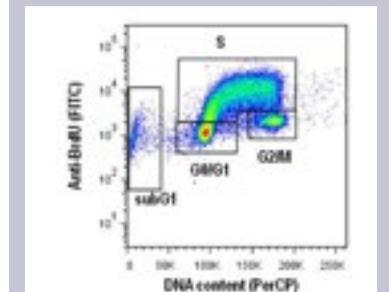


The BrdUFlowEx[®] FITC Kit enables also detection of cytokine secretion in context of cell proliferation. Human peripheral blood cells were stimulated for 2 days with PHA (5 μ g/ml) and for the last 6 hours were incubated with BrdU (10 μ M) and Brefeldin A (10 μ g/ml). Analysis of CD4 (PerCP), CD71 (APC), IFN-gamma (PE-Cy[™]7) and BrdU (FITC) was performed.

The image shows IFN-gamma and BrdU signal in CD4+ cells. (Antibodies to CD4, CD71, and IFN-gamma are not included in the kit).



Cell cycle analysis of Jurkat cells cultured for 1 hour in presence of 10 μ M BrdU. Total DNA (7-AAD signal) was detected in PerCP channel. Cells in G0/G1 phase, S phase, G2/M phase, and a subpopulation of cells with lower DNA content (probably apoptotic cells) are marked.



CellCycleFlowEx® Kit

Catalog No.: **ED7069**
 Regulatory status: **RUO**
 Quantity: **200 tests**

Reagents provided: **Propidium iodide**
RNAse A
10× Wash Buffer

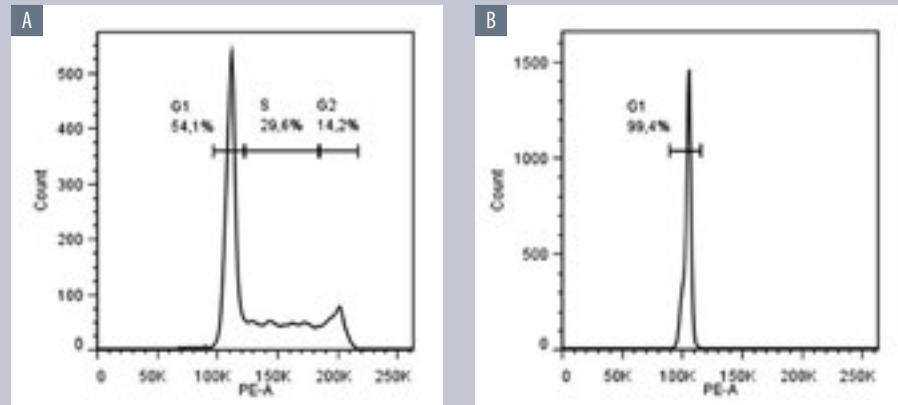
CellCycleFlowEx® Kit is intended for cell cycle analysis based on measuring of DNA content using flow cytometry. It enables to recognize the cells in G0/G1 phase (the same amount of DNA as the resting cells), S phase (in the process of DNA duplication), and G2/M phase (having double the amount of DNA as the

resting cells). The kit is suitable for testing suspensions of isolated cells, such as leukocytes isolated from peripheral blood (PBMC) or cells from tissue culture.

This product was developed with the support of the Technology Agency of the Czech Republic (Project TA03010719).

Example Data

Analysis of A) PBMC stimulated with PHA (5 µg/ml) for 3 days and B) unstimulated control cells. Distribution of cells in the singlet gate according to the DNA content into the G0/G1, S and G2/M phases of the cell cycle.



CellCycleFlowEx Kit®

Ki-67 Antibody

Regulatory status: **RUO**

Antigen	Clone	Host	Isotype	Reactivity	Application
Ki-67	Ki-67	Ms	IgG1	Hu, Bov	FC, WB, IHC, ICC

Formats: **Purified** | **Alexa Fluor® 488** | **PE** | **Alexa Fluor® 647** | **PE-Cy™7**

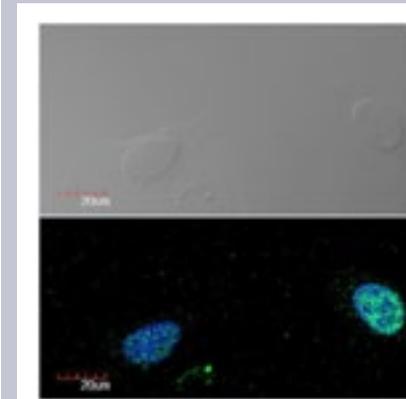
For currently available formats of this antibody see particular product page at our web www.exbio.cz.

Ki-67 is a highly protease-sensitive nuclear protein expressed in two isoforms (345 kDa and 395 kDa), both of which are identified by the antibody clone **Ki-67**. The **Ki-67** antigen is essential for cell proliferation and its expression is restricted to the cycling cells. It is detected in G1, S, G2 and M phase,

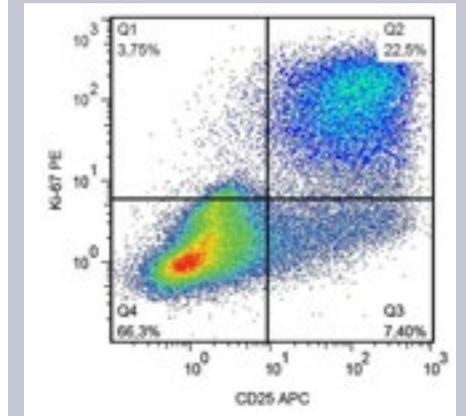
whereas it is absent in cells which are in G0 phase, and it is not associated with DNA repair processes. **Ki-67** thus represents an important tool for detection of proliferating cells, which is of great importance in tumor diagnostics and is commonly used as a prognostic factor in cancer studies.

Example Data

Immunocytochemistry detection of Ki-67 in U2OS cell line (human osteosarcoma) using monoclonal antibody Ki-67 (green). Cell nuclei stained with DAPI (blue).



Flow cytometry analysis of human peripheral blood mononuclear cells stimulated with PHA. Surface staining of CD25 (clone MEM-181 APC) was followed by permeabilization and nuclear staining of Ki-67 (clone Ki-67 PE).



ApoFlowEx[®] FITC Kit and Accessory Reagents

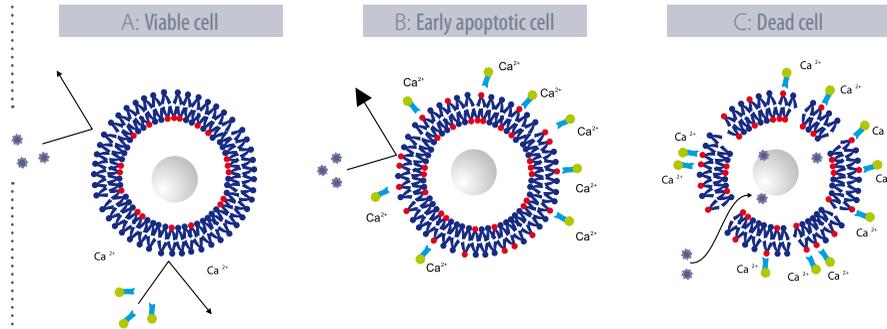
Catalog No.: **ED7044**
 Regulatory status: **RUO**
 Quantity: **100 tests**

Reagents provided: **Annexin V FITC**
Propidium iodide
Annexin V Binding Buffer

The **ApoFlowEx[®] FITC Kit** is intended for discrimination between early apoptotic, viable, and dead cells. Accessory reagents are available separately. One of the earliest indications of apoptosis is the translocation of phosphatidylserine from the inner to the outer leaflet of the plasma membrane. Once exposed to the extracellular environment, phosphatidylserine becomes available for annexin V, a 35–36 kDa Ca²⁺ dependent phospholipid-binding protein with a high affinity for it. The early apoptotic

cells have still intact plasma membrane, so their DNA is not accessible for propidium iodide dye, and these cells are annexin V positive and propidium iodide negative, unlike viable cells (annexin V negative, propidium iodide negative) and necrotic cells (annexin V positive, propidium iodide positive). To the latest group, however, belong also some late-apoptotic cells.

-  Phosphatidylserine (PS)
-  Annexin V – FITC
-  Propidium iodide (PI)

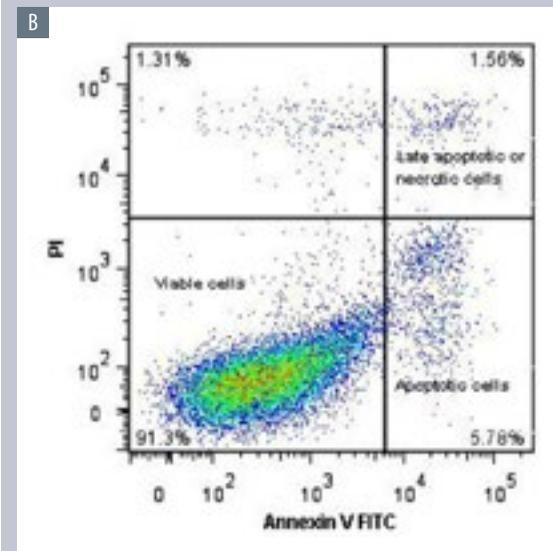
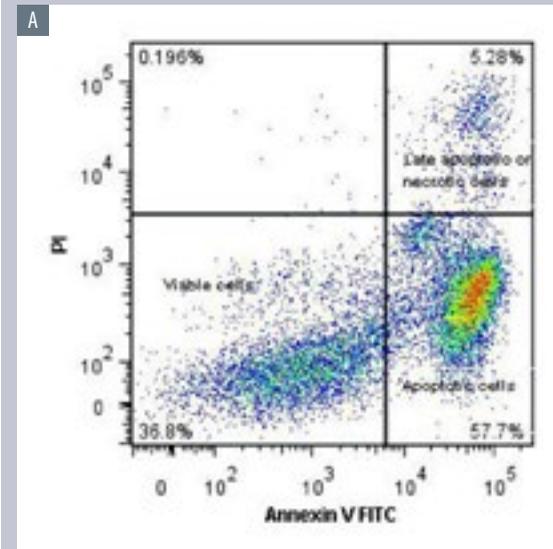


Accessory reagents

Catalog No.	Reagent	Quantity
EXB0024	Annexin V-FITC	100 tests
EXB0027	Annexin V-PE	100 tests
EXB0028	Annexin V-APC	100 tests
EXB0019	Annexin V Binding Buffer (10×)	50 ml of 10× concentrated buffer
EXB0018	Propidium Iodide	100 tests
EXB0018	7-AAD Viability Staining Solution	400 tests

Example Data

Analysis of camptothecin treated (A; apoptosis induced) and not treated cells (B) upon staining with ApoFlowEx[®] FITC Kit.



ApoFlowEx[®] FITC Kit

Apoptosis Related Antibodies

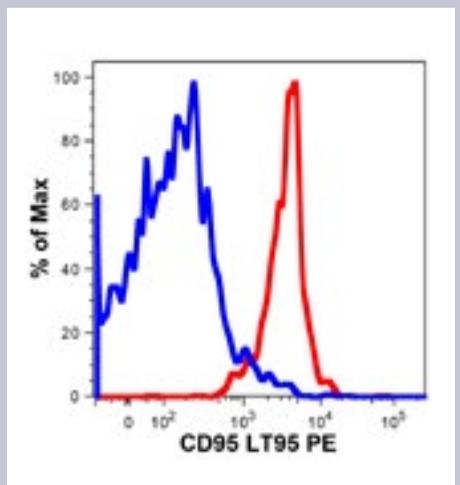
Regulatory status: RUO

Here we bring a list of our antibodies to apoptosis related antigens. For currently available formats of these antibodies see particular product pages at our web www.exbio.cz.

Antigen	Clone	Host	Isotype	Reactivity	Application
TRAIL / CD253	2E5	Ms	IgG1	Hu	FC, FUNC: blocking
TRAIL-R1 / CD261	DR-4-02	Ms	IgG1	Hu	FC, IP, ICC, FUNC: blocking or induction
TRAIL-R2 / CD262	DR5-01-1	Ms	IgG1	Hu	FC
TRAIL-R3 / CD263	TRAIL-R3-02	Ms	IgG1	Hu	FC
TRAIL-R4 / CD264	TRAIL-R4-01	Ms	IgG1	Hu	FC
DR6 / CD358	DR-6-04-EC	Ms	IgG1	Hu	FC, IP, ICC
Fas-L / CD178	NOK-1	Ms	IgG1	Hu	FC IP, WB, ICC, FUNC: blocking
Fas / CD95	LT95	Ms	IgG1	Hu	FC, IHC(P)
TWEAK / CD255	CARL-1	Ms	IgG3	Hu	FC, IHC, FUNC: blocking
TWEAK-R / CD266	ITEM-4	Ms	IgG2b	Hu	FC, WB, IHC(F), FUNC: blocking
Daxx / DAP6	DAXX-01	Ms	IgG1	Hu	WB, ICC
	DAXX-03	Ms	IgG1	Hu	IP, WB, ICC
DDIT4L	DDIT-03	Ms	IgG1	Hu	FC, WB
ARAP1 / centaurin	ARAP1-2	Ms	IgG1	Hu	IP, WB, ICC
Bcl2	Bcl2/100	Ms	IgG1	Hu	FC, IP, WB, IHC(P), IHC(F), ICC

Example Data

Analysis of CD8+ T lymphocyte subpopulations in human peripheral blood using CD95 (clone LT95) PE conjugated antibody. Red histogram shows central memory CD27+CD45RA- cells. Blue histogram shows naive CD27+CD45RA+ cells.



Quality statement

EXBIO Praha, a.s. is dedicated to its customers and to providing them with the products and services that are of the highest quality and that facilitate successful research. Our company has been assessed and certified as meeting the requirements of ISO 9001:2008, ISO 14001:2004, and ISO 13485:2003 for the following activities:

- Design, development and production of biotechnological products for research use.
- Design, development and production of *in vitro* diagnostics for cell and protein analysis.

Regulatory Notes

■ **CE-IVD** reagents are intended for *in vitro* diagnostic use in laboratories outside USA and Canada. These CE-IVD reagents conform to the European *In Vitro* Diagnostic Medical Device Directive 98/79/EC.



■ **RUO** reagents are intended for research use only. Not for use in diagnostic or therapeutic procedures.



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