The Dead Cell Removal Kit has been developed for fast and easy elimination of dead cells, dying cells and debris from cell culture or tissue preparations, by using Dead Cell Removal MACS MicroBeads.

Application of Dead Cell Removal Kit: Improvement in Immunocytochemical Staining of MACS selected Breast Cancer Cells from cryopreserved samples

By courtesy of Dr. N. Gachard and Mrs A. Allegraud, Laboratoire d´Hématologie, Hopital Universitaire Dupuytren, 2, Avenue Martin-Luther-King, 87042 Limoges, France.

Application

The detection of small numbers of circulating tumor cells and micrometastases has become increasingly important in breast cancer for determining prognosis and therapeutical applications. The conventional technique for such detection is immunocytochemical staining (ICC). The combination of immunomagnetic enrichment of circulating carcinoma cells by using for example MACS MicroBeads directed to Human Epithelial Antigen (HEA) and subsequent ICC, allows a highly sensitive detection.

We used cryopreserved samples of patients with known clinical outcomes. The thawing procedure increases the number of dead cells in the sample thus lowering the quality of ICC detection (Fig. 1). Therefore dead cells are depleted by using the MACS Dead Cell Removal Kit (Fig. 2). The procedure is based on labeling apoptotic cells, dead cells and debris with paramagnetic MACS Dead Cell Removal MicroBeads and the subsequent retention of the unwanted material in the magnetic field.

Material and Methods

The cryopreserved cell sample was thawed, washed twice in PBS and resuspended in 100µl of Dead Cell Removal MicroBeads per 1×10^7 total number of cells. After incubation of the cells with the paramagnetic MACS MicroBeads for 15 minutes at room temperature, the mixture was applied onto an MS column, which was placed in the magnetic field by using the MiniMACS separation unit. Magnetically labeled apoptotic cells, dead cells and debris were retained in the magnetic field. After washing the columns with binding buffer, the non-labeled fraction was eluted and collected as live cell fraction.

The subsequent positive immunomagnetic selection of disseminated breast cancer cells was performed by using the MACS HEA MicroBeads, according to the MACS standard protocol.

After cytospin preparation, the alkaline phosphatase antialkaline phophatase (APAAP) technique was applied by using BerEP4 monoclonal antibody, according to the manufacturer´s instruction (Fig. 3).

Results

The majority of the dead cells were depleted from defrosted cryopreserved samples by using the MACS Dead Cell Removal Kit. In a series of three experiments, a depletion rate of 45% to 90% dead cells was achieved. The mortality before and after depletion of dead cells was microscopically evaluated with trypan blue staining.

Removing dead cells prior to the immunomagnetic isolation of carcinoma cells and subsequent immuncytochemical staining allowed the unspecific background to be reduced as shown in Fig 3.

In conclusion, depletion of dead cells with the MACS Dead Cell Removal Kit improved the quality of the immunochemical staining of enriched breast cancer cells.

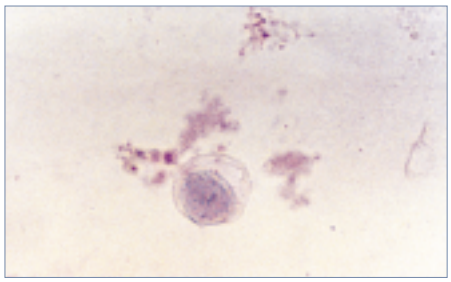

Fig. 1: Thawed sample of cryopreserved peripheral blood sample before removal of dead cells and debris with the MACS Dead Cell Removal Kit.

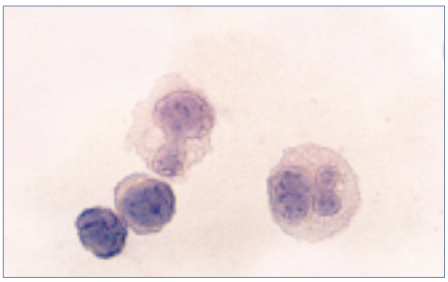

Fig. 2: Thawed sample of cryopreserved peripheral blood sample after removal of dead cells.

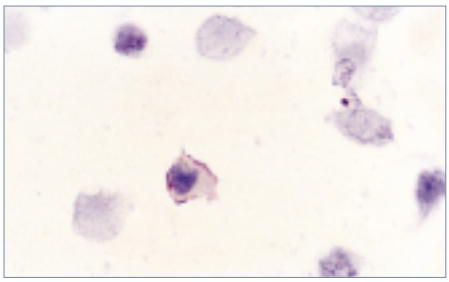

Fig. 3: HEA positive cell after immunomagnetic isolation, cytospin and ICC with BerEP4 mAB.

Dead Cell Removal Kit # 130-090-101

Related Products

Human Epithelial Antigen (HEA) MicroBeads	# 130-061-101
MiniMACS	# 130-042-101
MS Columns	# 130-042-201

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone: +49 - 2204 - 83060
FAX: +49 - 2204 - 85197

Miltenyi Biotec Inc.
12740 Earhart Avenue
Auburn, CA 95602, USA
Phone: 800 FOR MACS
530 888 - 8871
FAX: 530 888 - 8925

Miltenyi Biotec U.K.
Phone: 01483 - 799800
FAX: 01483 - 799811
Miltenyi Biotec France
Phone: 01 56 98 16 16
FAX: 01 56 98 16 17

Miltenyi Biotec Italy
Phone: 051 22 67 01
FAX: 051 23 46 28
Miltenyi Biotec Spain
Phone: 91 41 10 162
FAX: 91 56 12 987

Miltenyi Biotec
www.MiltenyiBiotec.com

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