APPENDIX

Appendix A: Reagent and buffer preparation

1. Reagents for cell culture

1.1 RPMI 1640 medium

RPMI 1640 powder (GibcoBRL)	1	pack
	10.4	g
$NaHCO_3$	2	g
Streptomycin (0.2 g/ml)	500	µl
(M & H MANUFACTURING CO.,LTD.)		
Penicillin ($1x10^6$ U/ml)	100	µl
(M & H MANUFACTURING CO.,LTD.)		
HEPES buffer (5M) (Sigma)	5	ml
Dissolved in de-ionized H_2O and adjust		
volume to 1000 ml and pH to 7.2		
Filtered through 0.2 µm millipore membrane filter		
then added Fungizone (5 mg/ml) (Bristol-Myers)	50	µl
and stored at 4°C		

1.2 Complete culture medium

RPMI 1640 medium	90	ml
Fetal bovine serum (FBS) (biowest)	10	ml

1.3 Freezing medium

DMSO (Sigma)	10	ml
FBS (biowest)	90	ml

Mixed well and stored at 4°C

Medium should be freshly prepared before use

2. Reagents for T cells isolation

2.1 1X Phosphate buffer saline (PBS) pH 7.2

NaCl	8.00	g
KCl	0.20	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.20	g

Dissolved in de-ionized water 1000 ml

and adjusted pH to 7.2

Filtered through 0.2 μm millipore membrane filter

and stored at 4°C

2.2 Separating buffer (0.5%BSA and 2mM EDTA in PBS pH7.2)

Bovine serum albumin (Sigma)	5	g
1M EDTA	2	ml

Dissolved in 1X PBS pH 7.2 to 1000 ml

Filtered through 0.2 μm millipore membrane filter

and stored at 4°C

3. Reagents for T cell proliferation assay and intracellular cytokine assays

3.1 Staining buffer (1%BSA and 0.1%NaN_3 in PBS pH 7.2)

Bovine serum albumin	5	g
NaN_3 (MERCK-SCHUCHARDT)	0.5	g

Dissolved in 1X PBS pH 7.2 to 500 ml

Filtered through 0.2 μm millipore membrane filter

and stored at 4°C

3.2 Cytokine staining buffer (1%BSA, 0.1%NaN_3 and 0.1% saponin, PBS pH 7.2)

Bovine serum albumin	5	g
NaN_3	0.5	g
Saponin (Sigma)	0.5	g

Dissolved in 1X PBS pH 7.2 to 500 ml

Filtered through 0.2 μm millipore membrane filter

and stored at 4°C

3.3 4% Paraformaldehyde

Paraformaldehyde (Sigma)	20	g

Dissolved in 1X PBS pH 7.2 to 500 ml

Filtered through 0.2 μm millipore membrane filter

and stored at 4°C.

Appendix B: Product description

1.1 Recombinant human GM-CSF

AbD serotec
A Division of MorphoSys

Datasheet: PHP141

Description:	RECOMBINANT HUMAN GM-CSF
Specificity:	GM-CSF
Format:	Rec. Protein
Product Type:	Recombinant Protein
Quantity:	10 µg

Product Details

Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information.

	Yes	No	Not Determined	Suggested Dilution
ELISA			▪	
Functional Assays	▪			

Where this protein has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only.

Target Species

Human

Product Form

Purified recombinant protein - lyophilised

Reconstitution

Reconstitute with 10 ul distilled water

Note

Care should be taken during reconstitution as the protein may appear as a film at the bottom of the vial. Serotec recommend that the vial is gently mixed after reconstitution.

Preparation

Recombinant protein prepared from *E.coli*

Preservative Stabilisers

None present

Endotoxin Level

<0.1 ng/ug

Approx. Protein Concentrations

1.0 mg/ml

Specificity

Human Granulocyte colony stimulating factor (GM-CSF) is a 14.6kD glycoprotein, which is produced by T cells, macrophages, fibroblasts and endothelial cells. GM-CSF is involved in the stimulation of proliferation and differentiation of granulocyte and macrophage progenitor cells.

PHP141 has an ED_{50} <0.1ng/ml, as determined by the dose dependant stimulation of the proliferation of

	human TF-1 cells.
Protein Molecular Weight	14.6kD (128 amino acid sequence/residues)
Purity	>98% by SDS PAGE/HPLC analysis
Storage	Prior to reconstitution store at +4°C. Following reconstitution store at -20°C. This product should be stored undiluted. Storage in frost-free freezers is not recommended. Avoid repeated freezing and thawing as this may denature the protein. Should this product contain a precipitate we recommend microcentrifugation before use.
Shelf Life	3 months from date of reconstitution.
Health And Safety Information	(A full Health and Safety assessment is available upon request)

For research purposes only, unless otherwise specified in writing by AbD Serotec.

'M52910:070926'

AbD Serotec Offices contact details click here

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Printed on 04 Jan 2008

1.2 Recombinant human IL-4

AbD serotec
A Division of MorphoSys

Datasheet: PHP044A

Description:	RECOMBINANT HUMAN INTERLEUKIN-4
Specificity:	IL-4
Format:	Rec. Protein
Product Type:	Recombinant Protein
Quantity:	2 µg

Product Details

Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information.

	Yes	No	Not Determined	Suggested Dilution
ELISA			▪	
Functional Assays	▪			0.1ng/ml - 10ng/ml

Where this protein has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own system using appropriate positive/negative controls.

Target Species

Human

Product Form

Purified recombinant protein - lyophilised

Reconstitution

Reconstitute with 0.02 ml distilled water

Note

Care should be taken during reconstitution as the protein may appear as a film at the bottom of the vial. Serotec recommend that the vial is gently vortexed after reconstitution and microcentrifuged before use.

Preparation

Purified recombinant IL-4 expressed in *E. coli.*

Preservative Stabilisers

None present

Endotoxin Level

<0.1 ng/ug

Approx. Protein Concentrations

0.1 mg/ml

Specificity

Interleukin-4 is a potent lymphoid cell growth factor, which stimulates the growth and survivability of B and T lymphocytes. This batch has an ED50 of <0.2ng/ml in a proliferation assay using human TF-1 cells.

Protein Molecular Weight	14.9kD (129 amino acid sequence)
Activity	>5 x 10^6 units/mg
Purity	>98% by SDS PAGE and HPLC
Storage	Prior to reconstitution store at +4°C. Following reconstitution store at -20°C. This product should be stored undiluted. Storage in frost-free freezers is not recommended. Avoid repeated freezing and thawing as this may denature the protein. Should this product contain a precipitate we recommend microcentrifugation before use.
Shelf Life	3 months from date of reconstitution
Health And Safety Information	(A full Health and Safety assessment is available upon request)

For research purposes only, unless otherwise specified in writing by AbD Serotec.

'M54997:070926'

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Printed on 04 Jan 2008

1.3 Carboxyfluorescein diacetate, succinimidyl ester (CFSE)

Material Safety Data Sheet

Revision Number: 1.2
Revision Date: 28-Mar-2006

Product and Company Identification

Product Name: 5(6)-CFDA, SE; CFSE (5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester) *mixed isomers*
Catalog Number: C1157
Unit Size: 25 mg
Manufacturer/Supplier: Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402-9132, USA
For US and Canada, Toll-Free Phone: 1-800-438-2209 · Fax: 1-800-438-0228
Phone: (541)465-8300 · Fax: (541)335-0305 · Web: http://probes.invitrogen.com
Technical Assistance: (541)335-0353 · E-mail: probestech@invitrogen.com

Composition / Information on Ingredients

5(6)-CFDA, SE; CFSE (5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester) *mixed isomers*

Molecular Formula: $C_{29}H_{19}NO_{11}$
Molecular Weight: 557.47
CAS Number/Name: 150347-59-4 / 2,5-Pyrrolidinedione, 1-[[[3',6'-bis(acetyloxy)-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5(or 6)-yl]carbonyl]oxy]-

Hazards Identification

Emergency Overview: Caution - substance not yet fully tested. To our knowledge, the hazards of this material have not been thoroughly investigated. We recommend handling all chemicals with caution.

Potential Health Effects
Inhalation: Not determined
Ingestion: Not determined
Skin: Not determined
Eyes: Not determined
Chronic Exposures: Not determined
Target Organs: Not determined

First Aid Measures

Potentially harmful. Avoid prolonged or repeated exposure. Wash thoroughly after handling. If eye or skin contact occurs, wash affected area with water for 15 minutes and seek medical advice. If inhaled, move individual to fresh air and seek medical advice. If swallowed, seek medical advice.

Fire Fighting Measures

Use dry chemical powder or appropriate foam extinguisher.

Accidental Release Measures

Use appropriate protective equipment and methods to clean up spilled substances promptly. Absorb spill onto an appropriate material. Collect and dispose of all waste in accordance with applicable laws.

Handling and Storage

Desiccation recommended. Store at <= -20°C.

Exposure Controls / Personal Protection

Wear appropriate gloves, protective clothing and eyewear and follow safe laboratory practices.
ACGIH/OSHA Permissible Exposure Limit Data: Not determined

Physical and Chemical Properties

Form:	Solid
Odor:	Not determined
Solubility in Water:	Low
Specific Gravity:	Not determined
pH:	Not determined
Boiling Point:	Not determined
Melting Point:	Not determined
Flash Point:	Not determined
Vapor Pressure:	Not determined

Stability and Reactivity

Thermal Decomposition: No decomposition if used according to specifications.
Dangerous Reactions: No dangerous reactions identified.
Dangerous Products of Decomposition: No dangerous decomposition products identified.

Toxicological Information

RTECS Number:	None known
Toxicity:	We are not aware of any toxicity data for this product.
Health Hazards:	See Potential Hazards below.
Potential Hazards:	The complete properties have not been investigated; however, similar compounds are known to be chemically reactive with proteins and other biochemicals and should be treated as potentially hazardous.
Carcinogenicity:	Not listed by NTP, IARC or OSHA.

Ecological Information

Do not allow product to reach ground water, water course, or sewage system.

Disposal Considerations

Consult local, state or national regulations for proper disposal.

Transport Information

Hazard Class: Not determined
Identification Number: Not determined
Packing Group: Not classified
Proper Shipping Name (Technical Name): Not determined

Regulations

US Toxic Substances Control Act (TSCA): Not listed
US Other: Not applicable
EEC EINECS Number: Not identified
EEC Risk Statements: Not determined
Other Country Regulations: None identified

Other Information

This material is sold for research purposes only and is not required to appear on the TSCA inventory. It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. Molecular Probes shall not be held liable for any damage resulting from handling or contact with the above product.

1.4 Pan T cell Isolation Kit II

MACS

Magnetic cell sorting

Pan T Cell Isolation Kit II
human

Order No. 130-091-156

Index

1. Description

Components

1 mL Pan T Cell Biotin-Antibody Cocktail:
Cocktail of biotin-conjugated monoclonal antibodies against CD14, CD16, CD19, CD36, CD56, CD123 and Glycophorin A.

2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to a monoclonal anti-biotin antibody (clone: Bio3-18E7.2; mouse IgG1).

Size
For 10^9 total cells, up to 100 separations

Product format
The Biotin-Antibody Cocktail is supplied in a solution containing 0.1% gelatine and 0.05% sodium azide.

The Anti-Biotin MicroBeads are supplied as a suspension containing 0.05% sodium azide.

Storage
Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

Using the Pan T Cell Isolation Kit II, human T cells are isolated by depletion of non-T cells (negative selection). Non-T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. In between the two labeling steps no washing steps are required. The magnetically labeled non-T cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled T cells pass through the column.

1.2 Background and product applications

The Pan T Cell Isolation Kit II is an indirect magnetic labeling system for the isolation of untouched T cells from human peripheral blood mononuclear cells (PBMC). Non-T cells, i.e. B cells, NK cells, dendritic cells, monocytes, granulocytes and erythroid cells, are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies against CD14, CD16, CD19, CD36, CD56, CD123 and Glycophorin A, and Anti-Biotin MicroBeads. Isolation of highly pure T cells is achieved by depletion of magnetically labeled cells.

Examples of applications

- Functional studies on T cells in which effects due to antibody-cross-linking of cell surface proteins should be avoided.
- Studies on signal requirements for T cell activation, induction of T cell proliferation, induction of T cell anergy, etc.
- Studies on signal transduction in T cells.
- Studies on regulation of T cell cytokine expression.

1.3 Reagent and instrument requirements

- Buffer (degassed): PBS (phosphate buffered saline) pH 7.2, supplemented with 0.5% BSA (bovine serum albumin) and 2 mM EDTA. Keep buffer cold (4–8 °C).

▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators:
  Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	max. number of labeled cells	max. number of total cells	Separator
MS	10^7	$2x10^8$	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	$2x10^9$	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	$2x10^{10}$	SuperMACS
autoMACS	$2x10^8$	$4x10^9$	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS or SuperMACS. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated antibodies (e.g. CD3-FITC # 130-080-401, CD2-PE # 130-091-115; Anti-Biotin-PE # 130-090-756, Anti-Biotin-APC # 130-090-856).
- (Optional) Pre-Separation Filter (# 130-041-407).
- (Optional) PI (propidium iodide) or 7-AAD for the flow cytometric exclusion of dead cells.

130-000-868.02

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2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMC should be isolated by density gradient centrifugation (see "General protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200xg for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with lymphoid tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation (e.g. Ficoll-Paque®) or the Dead Cell Removal Kit (# 130-090-101).

2.2 Magnetic labeling

▲ Work fast, keep cells cold, use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with less than 10^7 cells, use same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for $2x10^7$ total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filter # 130-041-407) to remove cell clumps which may clog the columns.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300xg for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 minutes at 4–8 °C.
6. Add 30 µL of buffer per 10^7 total cells.
7. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
8. Mix well and incubate for an additional 15 minutes at 4–8 °C.
9. Wash cells with buffer by adding 10–20 x labeling volume and centrifuge at 300xg for 10 minutes. Pipette off supernatant completely.
10. Resuspend up to 10^8 cells in 500 µ L of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation.

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total cells (see table in section 1.3).

Magnetic separation with MS and LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
   Allow the cells to pass through and collect effluent as fraction with unlabeled cells, representing the enriched T cell fraction.
4. Wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3x500 µL LS: 3x3 mL
   Collect entire effluent in the same tube as effluent of step 3. This fraction represents the enriched T cells.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-T cells.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the "XS Column data sheet".

Magnetic separation with the autoMACS™ Separator

▲ Refer to the "autoMACS User Manual" for instructions on how to use the autoMACS™ Separator.

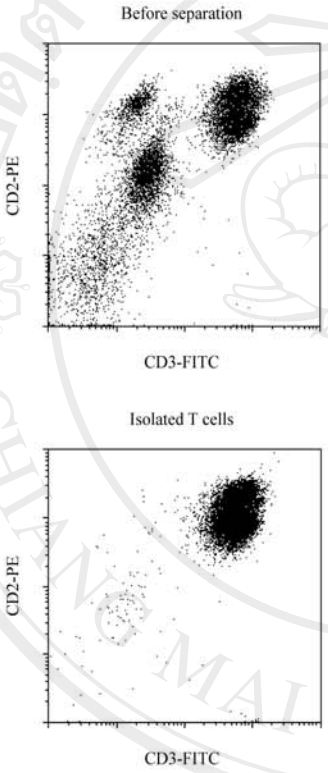
1. Prepare and prime the autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose program "Deplete".
3. Collect the negative fraction (outlet port "neg1"). This fraction represents the enriched T cells.
4. (Optional) Collect positive fraction (outlet port "pos1"). This fraction represents the magnetically labeled non-T cells.

2.4 (Optional) Evaluation of T cell purity

The purity of the enriched T cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody against a T cell marker, e.g. CD3-FITC (# 130-080-401), as recommended in the respective data sheet. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-T cells with the Biotin-Antibody Cocktail can be visualized by counter-staining with fluorochrome-conjugated anti-biotin antibodies, e.g. Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

3. Example of a separation using the Pan T Cell Isolation Kit II

Isolation of untouched T cells from PBMC using the Pan T Cell Isolation Kit II and an LS Column. Cells are fluorescently stained with CD3-FITC (# 130-080-401) and CD2-PE (# 130-091-115). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



Warning

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. MILTENYI BIOTEC GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. MILTENYI BIOTEC GmbH's liability is limited to either replacement of the products or refund of the purchase price. MILTENYI BIOTEC GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Ficoll-Paque® is a trademark of Amersham Biosciences UK Limited, Buckinghamshire, England.

MACS® is a registered trademark of Miltenyi Biotec GmbH.

1.5 MS separation columns

MACS

Separation Columns

MS Separation Columns

25 MS Separation Columns Order No. 130-042-201

Contents

25 MACS High Gradient Magnetic Separation Columns type MS and plungers for the elution of positively selected material, sterile packed. Capacity: max. 2×10^8 total cells and 10^7 magnetically labeled cells.

Applications

- ▲ Positive selection of up to 10^7 **positive** cells labeled with MACS MicroBeads from up to 2×10^8 **total** cells.
- ▲ Positive selection of rare cells from up to 2×10^8 **total** cells, e.g. antigen-specific B cells, $CD34^+$ cells and carcinoma cells from peripheral blood.
- ▲ Depletion of strongly magnetically labeled cells.
- ▲ Magnetic separation of biological material labeled with MACS MicroBeads such as bacteria, viruses, protozoa, cell organelles, etc.

Storage of MACS Separation Columns

Store columns dry, protected from light. Do not use after expiry date.

Instrument and reagent requirement

Magnetic Separator MiniMACS; MACS MicroBeads for magnetic labeling of cells.
Buffer: phosphate buffered saline (PBS) supplemented with 2 mM EDTA and 0.5 % bovine serum albumin (BSA).

How to use MACS MS Separation Column

MS Separation Columns have been developed for positive selection of human and animal cells, especially rare cells, out of a heterogeneous cell suspension in combination with the MiniMACS. They can also be used to separate other biological material such as plant cells, bacteria, viruses, protozoa, cell organelles and proteins.
The column has a hydrophilic coating that allows rapid filling. The recommended buffer is PBS supplemented with EDTA and BSA, but also other running buffers may be used with the MS Separation Column. The suitability of a specific buffer has to be tested experimentally.
The column is washed with buffer before separation. The material to be separated should be well suspended and should not contain clumps, aggregates or particles >30 µm. Clumps and aggregates can be removed using Pre-Separation Filters (# 130-041-407). After passing the material through the column, the retained material is washed with buffer to remove non-labeled material. To elute the positively selected material, e.g. bound cells, the column is removed from the magnet and the material is eluted in buffer with the plunger supplied.
MS Separation Columns can also be used for depletion of cells which strongly express the magnetically labeled surface antigen.

Protocol for cell separation using MS Separation Columns and MiniMACS

Preparation of the MS Separation Column

- Attach MiniMACS Separation Unit to the MACS MultiStand.
- Place the MS Separation Column in the MiniMACS Separation Unit. Place a collection tube under the column (see Figure).
- Apply 500 µl of degassed buffer on top of the column and let the buffer run through. Columns are "flow stop" and do not run dry. Then discard effluent and change collection tube. The unit is now ready for separation.

Set-up

Assembly

130-000-126.04

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Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

Order No. 130-042-201

Magnetic separation using MS Separation Columns

- Pipette magnetically labeled cell suspension containing up to 10^7 positive cells in maximum 2×10^8 total cells onto the column (up to 10^8 cells per 500 µl of buffer). Allow the cell suspension to run through and collect effluent as negative fraction.
- Wash column with 3 x 500 µl buffer and collect total effluent as negative fraction.
- Remove column from separator and place the column on a new collection tube.
- Apply 1 ml of buffer to the reservoir of the column and firmly flush out cells using the plunger supplied.
- (Optional) Separate positive fraction over a new column to increase the purity.

Important notes

▲ Use degassed buffer only! Degas buffer by applying vacuum, preferentially with buffer at room temperature. Excessive gas in running buffer will form bubbles in the matrix during separation. This may lead to clogging of the column and decrease the quality of separation.

▲ The recommended buffer is PBS supplemented with 2 mM EDTA and 0.5 % BSA. Different buffers may be used, but have to be tested experimentally.

▲ The columns are for single use only. The columns contain a biocompatible hydrophilic coating. This coating is washed out during the filling process.Use column immediately after filling to avoid formation of air bubbles caused by warming up.

▲ Use a maximum cell concentration of 10^8 cells per 500 µl of buffer when applying cells to the column.

▲ The time for filling the column with buffer is dependent on the storage conditions, temperature and humidity. Therefore, the time may vary from a few seconds to several minutes. This filling time has no influence on the quality of the separation.

▲ Do not use samples or buffers with too high a viscosity or with particles >30 µm.

▲ MS Separation Columns are not suitable for particles larger than 30 µm. To remove clumps and prevent aggregates in sample, resuspend material carefully and pass through 30 µm nylon mesh or Pre-Separation Filter (# 130-041-407) before separation. Wet filter with buffer before use.

▲ To increase purity, cells can be passed over a new, freshly prepared column a second time.

▲ For details on magnetic labeling, see "MACS Reagent Data Sheets".

▲ If the flow stops during separation, check that the buffer has been properly degassed. Start flow again with a slight push of the plunger. Do not pass the cells through the column with the plunger.

▲ When working with fresh anticoagulated blood or buffy coat, dilute before separation 1:2 with buffer.

▲ Do not use MS Separation Columns in combination with magnetic particles other than MACS MicroBeads. Magnetic forces in the column are very high and may damage biological material if other beads are used.

Technical specifications

- Typical capacity: 10^7 magnetically labeled lymphocytes. Column capacity may decrease to 3×10^6 magnetically labeled cells separating cells larger than lymphocytes.
- Recommended sample size for leukocytes: 10^4–10^7 labeled cells in 10^6–2×10^8 total cells. Sample concentration: up to 10^8 leukocytes/500 µl cell suspension.
- Typical enrichment rate: 50 up to 1000fold, depending on the strength and specificity of the magnetic labeling. Up to 10,000fold enrichment can be achieved by separation over two sequential columns.
- Void volume: 60 µl. Reservoir volume: 3.5 ml.
- Typical flow rate for PBS containing 0.5 % BSA: 0.35-0.5 ml/min.

Warranty

140-000-126-04

1.6 Brefeldin A

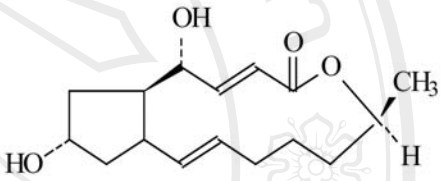
SIGMA

ProductInformation

BREFELDIN A
from Penicillium brefeldianum
Sigma Prod. No. B7651

CAS NUMBER: 20350-15-6

SYNONYMS: γ,4-Dihydroxy-2-[6-hydroxy-1-heptenyl]-4-cyclopentanecrotonic acid λ-lactone; cyanein; BFA



PHYSICAL DESCRIPTION:

Appearance: White powder
Molecular formula: $C_{16}H_{24}O_4$
Molecular weight: 280.4
E^M(215nm) = 11,300 (ethanol)[1,2]
Optical rotation: +96°± 2 (methanol)[1,2]
Melting point: 204°C ± 1°[1,2]

STORAGE / STABILITY AS SUPPLIED:

This product when stored sealed at 0-4°C showed no change by TLC in over 12 months.[2]

SOLUBILITY / SOLUTION STABILITY:

Sigma tests BFA for solubility in methanol at 10 mg/mL.[2] Stock solutions of Brefeldin A can be prepared in methanol (1 mg/mL)[3] or in ethanol (1 or 5 mg/mL)[3,5] and stored at -20°C.[4,5] Concentration can be verified by UV absorption (λ_{max} = 215 nm, log E^M = 4.05)[1]

GENERAL REMARKS:

Brefeldin A is a fungal metabolite (a macrocyclic lactone) which exhibits a wide range of antibiotic activity.[1,3] Brefeldin A (BFA) may be used to study cell processes which depend upon intracellular protein transport.

BFA reversibly inhibits the intracellular translocation of proteins in eukaryotes, e.g., during transport of proteins to the cell surface for secretion or expression.[3] Brefeldin A has been reported to block the response of cultured cells to cholera toxin.[5]

BFA inhibits protein synthesis in cultured cells[6] and inhibits the transport of secretory and lysosomal proteins at concentrations of 1-10 µg/mL.[7] "In HepG2 cells, BFA induces two blocks in the secretory pathway; one at the level of the endoplasmic reticulum-Golgi juncture and the other in the trans-Golgi network.

BREFELDIN A
from Penicillium brefeldianum
Sigma Prod. No. B7651

GENERAL REMARKS: (continued)

In contrast, transport from the Golgi complex to the lysosomes and from the plasma membrane to the lysosomes continued."[7] Vogel et al. also reported secretion blockage and redistribution of Golgi resident membrane proteins.[8]

Lippincott-Schwartz et al. reported on the effects of Brefeldin A (BFA) on the morphology and dynamics of endosomes, trans-Golgi network (TGN) and lysosomes. BFA treatment (at 5 μg/mL) induced changes in both the organization and distribution of the organellar components in all of these organellar systems.[9]

Brefeldin A was reported to enhance transcytosis of transferrin in cultured kidney cells.[10]

SYNTHESIS:

Total syntheses of (±) BFA and of (+) BFA have been reported.[11-13]

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