Product Insert: FerroSelect™ CD3/CD28 Activation & Expansion Kit - QP (Cat. 28-0013)

Doc: 26-0060-03 Eff. 15-Nov-2023

Product Description:

The guidelines below may be used for the selection of T cells from approximately 3.0 x 10^8 peripheral blood mononuclear cells (PBMC) followed by their subsequent activation and expansion. Alternatively, it may be used for the activation and expansion of T cells enriched through the depletion of unwanted cells using the AbsenT™ negative selection kits.

IMPORTANT NOTE: BioMagnetic Solutions uses fresh (non-frozen) cellular products for method development. Customers using frozen products such as cord blood for cell selection studies should develop their own procedures. Suggestions for using our products with frozen starting materials are available. Please contact us for assistance.

Sufficient materials are included in the kit to allow the selection of approximately 1.5 x 10^8 T cells.

BioMagnetic Solutions recommends that the user develops their own methodology for cell expansion and activation using the below as guidance, as different permutations and sources of media and/or reagents can impact the overall level of expansion and percentages of T cell subsets produced.

Product Contents	
Biotinylated anti-CD3 mAb	1 vial: 1.0 mL, 12 µg/mL in PBS w/ 1.0% rHSA.
Biotinylated Anti-CD28 mAb	1 vial: 1.0 mL, 12 µg/mL in PBS w/ 1.0% rHSA.
Streptavidin Ferrofluid (SA-FF)	1 vial: 1.0 mL, 75 µg/mL in 0.3% rHSA.
Storage: 2-8 ºC **Do Not Freeze**	Expiry Date: As per label/CoA

rHSA – recombinant Human Serum Albumin, mAb – Monoclonal Antibody

Additional Materials that have been used by BioMagnetic Solutions:

Cell Culture Medium

Sartorius: 4Cell® Nutri-T GMP xeno and serum free medium, Cat. No: 05-F3F2111-1K

OR

Fujifilm**:** Prime-XV T cell CDM xeno and serum free medium, Cat. No: 91154-1L

Recombinant Interleukin-2 (rIL-2): Akron, Cat. No: AK8223

Guidelines:

Details related to the selection of $CD3^+$ cells using either this kit or the FerroSelect™ CD3 Selection Kit - QP (Cat. 28-0003) can be found in the accompanying product insert.

Details related to the negative selection of $CD3^+$ cells through the removal of other cell types can be found in the product insert for the FerroSelect™ AbsenT™ CD3 Negative Selection Kit – QP (Cat. 28-0009).

The methods described below were developed by BioMagnetic Solutions as a guide for the end user. Cell preparation, activation, and expansion should be performed in a biosafety cabinet and sterile conditions maintained throughout. No animal products nor antibiotics were used for the activation/expansion of T cells.

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Expansion of T cells through Positive Selection employing Biotinylated Anti-CD3 and Streptavidin Ferrofluid.

1.0 Cell Isolation	
1.1	Cells should be selected using the conditions outlined in the product insert included for the positive selection of $CD3^+$ cells. Directly after selection these cells will have anti-CD3 and streptavidin on their surface. Activation occurs by the addition of the biotinylated anti-CD28 included in the kit. Alternatively, the end user may wish to add additional amounts of anti-CD3, anti-CD28 and streptavidin ferrofluid to the enriched T cell population, although this is not normally undertaken at BioMagnetic Solutions.
2.0 Cell Activation	
2.1	The CD3/CD28 Activation/Expansion Kit has been designed for use with up to 1.0×10^8 T cells (using maximum amount of anti-CD28 tested). After selection cells should be centrifuged at 200 x g for 10 minutes, the supernatant decanted, and the cell pellet resuspended in activation medium at a concentration of 5.0×10^7 cells/mL.
2.2	For the activation buffer, BioMagnetics Solutions have used either the Sartorius or Fujifilm medium supplemented with biotinylated anti-CD28 at a final concentration of 3.0 – 6.0 µg/mL.
2.3	Cells in the activation medium were incubated at 37 °C for 30 minutes in a humidified tissue culture incubator with 5% CO_2.
3.0 Cell Expansion	
3.1	After activation the cells were diluted to 5.0×10^5 – 1.0×10^6 cells/mL using either the Sartorius or Fujifilm medium containing Interleukin-2 (IL-2) at a final concentration of 10 – 20 ng/mL and incubated at 37 °C in a humidified tissue culture incubator with 5% CO_2.
3.2	When approaching confluency, cells should be diluted back to their original concentration in the medium containing IL-2. BioMagnetic solutions has continued to expand cells in this fashion for 14 days after initiating the culture.
3.3	Using the above approach, T cells normally continue to expand over a 14 day period, although by days 6 – 8 the immunocapture reagents and anti-CD28 cannot be identified on their surface.
4.0 Restimulation of T cells in Culture (Optional)	
4.1	Investigators have the option to restimulate cells at any point over the expansion period should they desire. This can be undertaken when the cells are becoming confluent by diluting them in either the Sartorius or Fujifilm medium containing anti-CD3 and anti-CD28 at a final concentration of 3.0 – 6.0 µg/mL and streptavidin ferrofluid at 20.0 µg/mL.

Expansion of T cells obtained by the removal of other cell types using BioMagnetic Solutions AbsenT CD3 kit.

1.0 Cell Isolation	
1.1	T cells should be enriched by the removal of other cell types from PBMCs using BioMagnetic Solutions AbsenT CD3 Negative Selection Kit. The T cells will not have any immunomagnetic selection reagents on their surface.
1.2	T cell activation occurs through the addition of anti-CD3 and anti-CD28 mAbs along with streptavidin ferrofluid to the cells in a defined medium.
2.0 Cell Activation	
2.1	The CD3/CD28 Activation & Expansion Kit has been designed for use with up to 1.0 x 10^8 T cells (using the maximum amount of anti-CD28 tested). After selection, cells should be centrifuged at 200 x g for 10 minutes, the supernatant decanted and the cell pellet resuspended in activation buffer at a concentration of 5.0 x 10^7 cells/mL.
2.2	For the activation buffer, BioMagnetics Solutions have used either the Sartorius or Fujifilm medium supplemented with biotinylated anti-CD3 and anti-CD28 at a final concentration of 3.0 – 6.0 µg/mL along with streptavidin ferrofluid at a final concentration of 20.0 µg/mL.
2.3	Cells in the activation medium should be incubated at 37 °C for 30 minutes in a humidified tissue culture incubator with 5% CO_2.
3.0 Cell Expansion	
3.1	After activation the cells were diluted to 5.0 x 10^5 – 1.0 x 10^6 cell/mL using either the Sartorius or Fujifilm medium containing Interleukin-2 (IL-2) at a final concentration of 10 – 20 ng/mL and incubated at 37 °C in a humidified tissue culture incubator with 5% CO_2.
3.2	When approaching confluency, cells should be diluted back to their original concentration in the starting medium containing IL-2. BioMagnetic Solutions has continued to expand cells in this fashion for 14 days after initiating the culture.
3.3	Using the above approach, T cells normally continue to expand over a 14 day period, although by days 6 – 8 the immunocapture reagents and anti-CD28 cannot be identified on their surface.
4.0 Possible restimulation of T cells in culture	
4.1	Investigators have the option to restimulate cells at any point over the expansion period should they desire. This can be undertaken when the cells are becoming confluent by diluting them in either the Sartorius or Fujifilm medium containing anti-CD3 and anti-CD28 at a final concentration of 3.0 – 6.0 µg/mL and streptavidin ferrofluid at 20 µg/mL.

Additional Catalog Items

BioMagnetic Solutions produces kits for the selection of $CD4^+$ and $CD8^+$ T cell subsets along with their counterparts for the enrichment of these cell types using AbsenT™ technology. Cells obtained using these kits may also be activated and expanded using this activation/expansion kit, although the conditions for use should be established by the end user.

Precautions and Disclaimers:

This product is for Research Use Only, not for use in Diagnostic Procedures, and for *ex vivo* use only. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices. The kit should not be used post expiration dating. There may be excess material in the vials due to the product specific requirements for use.

This product is manufactured in the USA entirely from material of non-animal origin. The manufacture, packaging, storage, and transportation of these materials do not involve the use of material of animal origin. This information is to be used for the purpose of determining animal origin only and not to be confused with 'country of origin' for import/export purposes.

Limited Use Label License:

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Certifications:

BioMagnetic Solutions' Quality Management System is certified to ISO 9001:2015 and ISO 13485:2016 by NQA.