iba
Solutions
For Life Sciences

Cell Selection &
Expansion

CELL SELECTION & EXPANSION

Traceless affinity enrichment for high-quality cell application

> Selection - Expansion - Staining
> All applications - one provider
> Cell isolation directly from whole blood or PBMCs
> Label-free, authentic cells

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Stratech

Order online: iba-lifesciences.com

IBA'S CELL SELECTION & EXPANSION PORTFOLIO

Cell separation of authentic cells from PBMCs and whole blood. No magnetic beads, no nanobeads, no high affinity antibodies!

Manual cell separation: Fab-TACS® Gravity

Manual chromatography columns allow the selection of specific cell populations without the need for any further equipment.

Cell Isolation → Cell Expansion → Cell Staining

Automated cell separation: FABian®

Automated cell separation

IBA's cell selection device ensures fully automatic enrichment of target cells in a highly reproducible manner.

It is a small bench top instrument which is used to select cells for up to 9 ml whole blood or other sources.

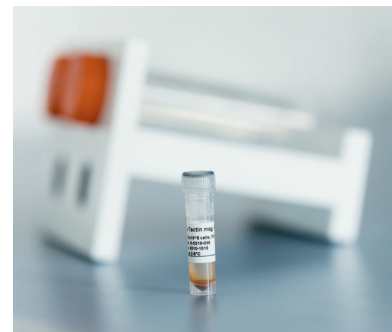Cell Isolation → Cell Expansion → Cell Staining

The Streptamer® technology covers a wide range of different cell applications such as cell isolation, cell expansion and cell staining.
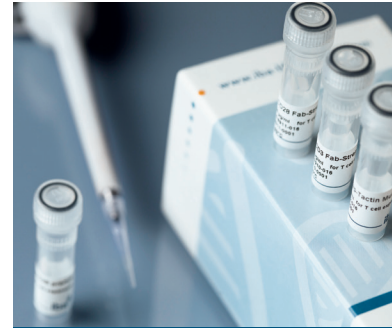
Magnetic cell separation for receptor-specific or antigen-specific T cells based on the fully reversible Streptamer® technology.

| Cell Isolation | → | Cell Expansion | → | Cell Staining |

Cell isolation via magnetic beads conjugated to Fab Streptamers

T cell activation, proliferation and differentiation with CD3/CD28 Streptamers. The fully removable reagents allow precise termination of the stimulation experiments.
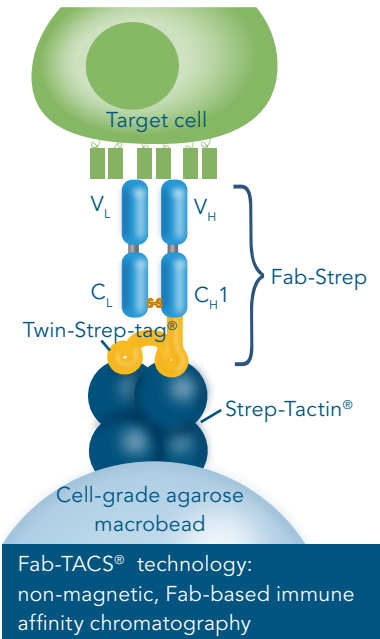
| Cell Isolation | → | Cell Expansion | → | Cell Staining |

T cell expansion with CD3/CD28 Streptamers

Receptor-specific T cell or antigen-specific T cell staining based on the fully reversible Streptamer® technology.

| Cell Isolation | → | Cell Expansion | → | Cell Staining |

Cell staining via fluorescence labeled MHC I Streptamers

Target cell

V_L V_H

C_L C_H1

Fab-Strep

Twin-Strep-tag®

Strep-Tactin®

Cell-grade agarose macrobead

Fab-TACS® technology:
non-magnetic, Fab-based immune
affinity chromatography

Fab-TACS® - TRACELESS AFFINITY CELL SELECTION

IBA's unique Strep-tag®/Strep-Tactin® system is the platform for the traceless affinity cell selection (Fab-TACS®). Fab-TACS® uses immune affinity chromatography based on CD-specific Fab-fragments for reversible capture and release of target cells. The innovative Fab-TACS® procedure delivers label-free, non-activated target cells in a standardized manner of highly reproducible quality. Targeted cell can be separated from PBMCs, whole blood or other blood preparations.

The Fab-TACS® positive selection avoids the use of high affinity antibodies whose usage causes unfavorable effects. This technique prevents strong and irreversible binding to cells or immune system components to the Fc region, cell stimulation as well as receptor blockade.

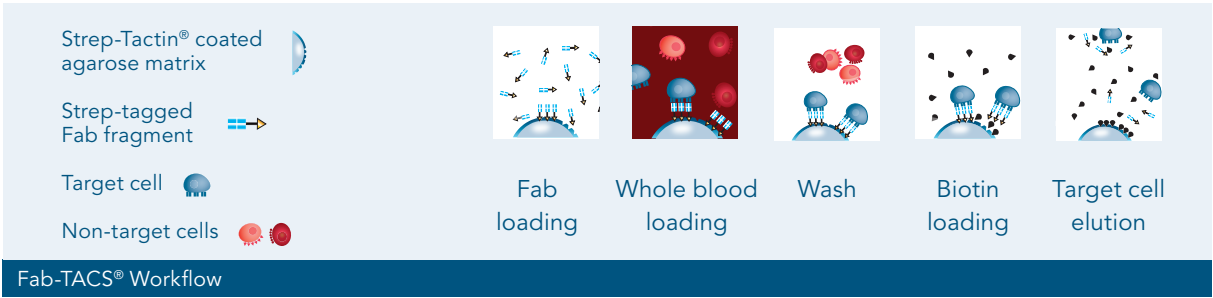### Manual and automated cell selection with Fab-TACS®

Application	Product
Manual Cell Selection	Fab-TACS® Gravity
Automated Cell Selection	FABian®

Key Features:
› From PBMCs or whole blood
› High purity
› High yield
› High viability
› Label-free cells

Simple Workflow

Columns for either Fab-TACS® Gravity or FABian® are filled with a Strep-Tactin® coated matrix made of cell-grade agarose. Strep-tagged low affinity Fab fragments (Fab-Streps) specifically bind to the matrix. Subsequently, PBMCs, whole blood or other blood preparations pass through the column. Target cells adhere to the matrix based on the exclusive binding of the Fab-Strep to the target cell. Non-target cells are efficiently washed away. In a final step, the addition of biotin causes the elution of the target cells and the Fab-Streps due to the higher affinity of biotin to Strep-Tactin®. After elution, the Fab-Streps self-dissociate from the cell surfaces. The label-free authentic target cells are now ready for further downstream applications.

Strep-Tactin® coated agarose matrix

Strep-tagged Fab fragment

Target cell

Non-target cells

Fab loading

Whole blood loading

Wash

Biotin loading

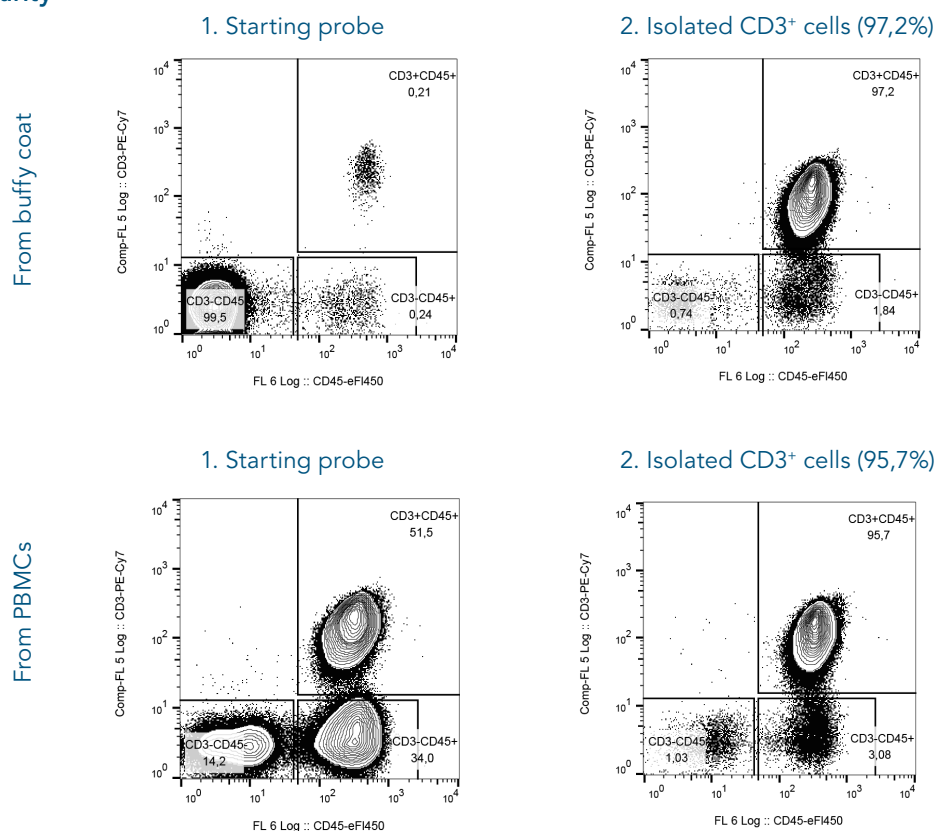Target cell elution

Fab-TACS® Workflow

Fab-TACS® is highly efficient

Depending on the source of extraction, common positive cell isolation technologies require different kits for the same antigen. Cell isolation from whole blood, buffy coat, PBMCs or single cell suspensions with the Fab-TACS® technology is possible with only one isolation kit.

Source	Yield	Purity	Viability
Buffy coat	98,2%	97,2%	98,7%
PBMCs	96,7%	95,7%	99,1%

Purity

From buffy coat

1. Starting probe

2. Isolated CD3⁺ cells (97,2%)

From PBMCs

1. Starting probe

2. Isolated CD3⁺ cells (95,7%)

The reversible Fab-TACS® technology provides non-activated cells

Fab-TACS® is a traceless affinity cell selection process, which works with low affinity Fab-Strep fragments and a Strep-Tactin® multimer. The Fab fragments are multimerized on Strep-Tactin® during the cell selection process. After addition of biotin, the Strep-Tactin® multimer is released from the Fab fragment. The Fab fragment then self-disso-ciates from the target cell due to its low affinity character. This allows the isolation of label-free and non-activated cells.

In comparison, cells purified with other positive cell selection tech-niques are labelled with high affinity antibodies. Those antibodies stick to the cells even after the selection process, which leads to an activation of the cells and influences downstream applications.
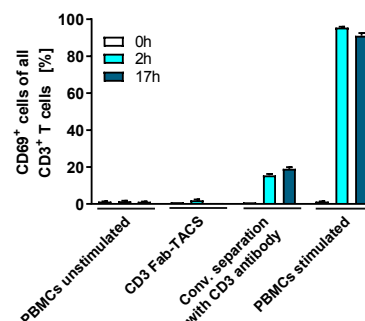
Fab-TACS® provides non-activated cells

MANUAL CELL SEPARATION - Fab-TACS® GRAVITY

The Fab-TACS® Gravity column is a common chromatography flow column filled with a Strep-Tactin® coated matrix made of cell-grade agarose.

Fab-TACS® Gravity columns

Key benefits
›   One kit for all sources: PBMCs, whole blood, buffy coat, single cell suspension
›   Easy and specific selection of target cells
›   High yield and high purity
›   Preserve authentic properties, full effector function and viability of selected cells
›   Magnet-free

Fab-TACS® Gravity - Introductory Kit
Cat. no. 6-3299-002

›   2x Fab-TACS® Gravity columns
›   Fab-Strep of choice
›   Buffer CI (10x)
›   Biotin Stock Solution

Fab-TACS® Gravity

Available isolation kits for Fab-TACS® Gravity

Species	Product description	Size	Cat. no.
human	CD3 T cells	4x kit 10x kit	6-3201-004 6-3201-010
	CD4 T helper cells and monocytes	4x kit 10x kit	6-3202-004 6-3202-010
	CD8 Cytotoxic T cells	4x kit 10x kit	6-3203-004 6-3203-010
	CD31 T cells	4x kit 10x kit	6-3216-004 6-3216-010
mouse	CD3 T cells	4x kit 10x kit	6-3404-004 6-3404-010
	CD4 T helper cells and monocytes	4x kit 10x kit	6-3401-004 6-3401-010

Fab-TACS® Gravity with adapter

Accessory

Product description	Size	Cat. no.
Fab-TACS® Gravity Adapter (for use with 50 ml Falcon tube)	1 adapter	6-3331-001

AUTOMATED CELL SEPARATION - FABian®

The FABian® cell selection device is a bench top instrument which automates the whole selection procedure based on IBA's Fab-TACS® technology. Similarly, the cells of interest can be isolated in high yields, high viability and purity from diverse sources such as whole blood, buffy coat or other cell suspensions.

Automation of the cell selection process secures highly reproducible results and also saves time, e.g. for PBMC preparation.

Key benefits
> Higly reproducible
> Preserve authentic properties, full effector function and viability of selected cells
> Automatic process - minimal hands-on time
> Embedded system, no external computer needed
> Magnet-free process

FABian®

FABian® device

Product description	Cat. no.
FABian® automated cell selection system	6-6100-260

Isolation kits for FABian®

Species	Product description	Size	Cat. no.
human	CD3 Isolation Kit	1 kit	6-6001-001
		10 kits	6-6001-010
	CD4 Isolation Kit	1 kit	6-6002-001
		10 kits	6-6002-010
	CD8 Isolation Kit	1 kit	6-6003-001
		10 kits	6-6003-010
	CD14 Isolation Kit	1 kit	6-6017-001
		10 kits	6-6017-010
	CD19 Isolation Kit	1 kit	6-6013-001
		10 kits	6-6013-010
	CD28 Isolation Kit	1 kit	6-6014-001
		10 kits	6-6014-010
	CD31 Isolation Kit	1 kit	6-6016-001
		10 kits	6-6016-010
	CD45RA Isolation Kit	1 kit	6-6007-001
		10 kits	6-6007-010
	CD81 Isolation Kit	1 kit	6-6015-001
		10 kits	6-6015-010
mouse	CD3 Isolation Kit	1 kit	6-6504-001
		10 kits	6-6504-010
	CD4 Isolation Kit	1 kit	6-6501-001
		10 kits	6-6501-010

FABian® cell selection kits
> Receptor-specific Fab-Strep
> Biotin for cell elution
> Cell isolation buffer
> Fab-TACS® auto column
> Tube and syringe set

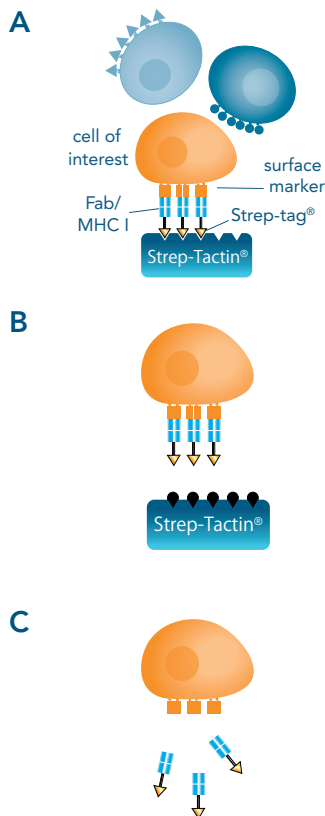Depending on the kit, up to 9 ml of whole blood or up to 6.25 ml buffy coat can be processed.

FABian® kit

STREPTAMER® TECHNOLOGY

Streptamer® technology
> Authentic cell populations
> Fully reversible mechanism
> Isolation, expansion, staining

The Streptamer® portfolio covers different stages in cell research from cell isolation to cell expansion and cell staining. Due to the completely reversible mechanism, which is based on IBA's proprietary Strep-tag® technology, serial selection cycles and expansion experiments are possible.

Application	Product
Cell isolation	T cell isolation with magnetic beads ❯ Fab-Streptamer® (receptor-specific) or ❯ MHC I Streptamer® (antigen-specific)
Cell expansion	CD3/CD28 Streptamer® for T cell expansion
Cell staining	❯ Fab-Streptamer® (receptor-specific) ❯ MHC I Streptamer® (antigen-specific)

A

cell of interest

surface marker

Fab/ MHC I

Strep-tag®

Strep-Tactin®

B

Strep-Tactin®

C

The Streptamer® principle
(A) Multimerization & binding of target cell
(B) After purification, release of Strep-Tactin® upon addition of biotin
(C) Self-dissociation of Fab or MHC-I fragments

The Streptamer® principle

Low affinity Strep-tagged Fab fragments or MHC molecules are employed to convey the specificity to the target cell population. In order to achieve a high binding affinity for proper cell isolation, expansion or staining, the Strep-tag® fragments bind to a multimerized Strep-Tactin®, a streptavidin derivative. Due to this a high binding avidity of the fragment is achieved. Strep-Tactin® is available as conjugate with a magnetic microbead for magnetic cell isolation, label-free for expansion experiments or conjugated with the fluorochromes APC or PE for cell staining or FACS isolation.

Upon addition of low concentrations of biotin, which competes with high affinity for the binding of Strep-tag® to Strep-Tactin®, isolation, expansion or staining reagents dissociate rapidly from the cell surface. This reversibility enables sequential positive cell selections with magnetic beads, accurate termination of stimulation events as well as multiple cell stainings with different Strep-Tactin® fluorochromes.

Key benefits

No high affinity antibody used! Fully reversible reagents:
> No isolation, expansion or staining reagents remain on the cells
> Avoid unspecific signaling events or stimulation of cells
> Preserve authentic properties, full effector function and viability of selected cells
> Serial positive selections of even rare cell subsets possible
> Accurate termination of stimulation

T CELL ISOLATION WITH MAGNETIC BEADS AND FAB STREPTAMER®

Receptor-specific cell selection

Fab Streptamer® reagents are used for receptor-specific cell isolation from PBMCs and subsequent cell staining of any cells carrying a surface marker such as CD3, CD4 etc.

Fab Streptamer® technology

Fab-Streptamer® comprise two components:
› Low affinity Fab, which is fused to Twin-Strep-tag® (Fab-Strep)
› Strep-Tactin® multimers labelled with a magnetic microbead

The Fab fragment confers the specificity to a certain CD marker receptor. The Twin-Strep-tag® binds to Strep-Tactin®, which is then used for isolation or staining of the target cell. For cell isolation the StrepMan magnet (cat.no 6-5650-065) is required. Upon addition of biotin, a competitive Strep-tag® ligand, the Strep-Tactin® multimer dissociates from the complex and also the Fab fragment will detach from the target cell.
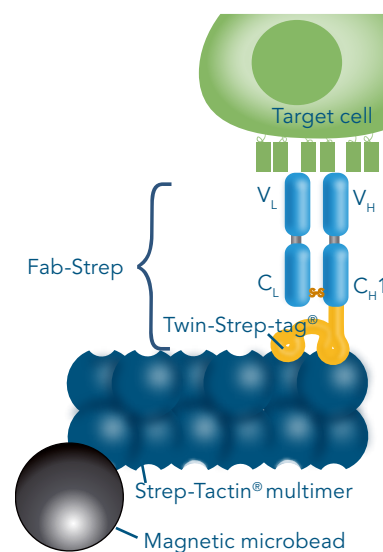
Cell isolation via magnetic beads conjugated to Fab Streptamer®

Key benefits

› Fully functional target cells
› Complete dissociation of isolation reagents from purified cells
› Serial positive selections of different markers allow isolation of even rare cell subsets
› High purity and recovery of the target cells

Fab Streptamer® Isolation Kits*

Species	Cell of Interest	Product description	Cat. No.
human	T cells	CD3$^+$	6-8000-201
	T helper cells and monocytes	CD4$^+$	6-8000-206
	Cytotoxic T cells	CD8$^+$	6-8000-203
	Naive T cells	CD4$^+$ CD45RA$^+$	6-8000-221
		CD8$^+$ CD62L$^+$ CD45RA$^+$	6-8000-219
	Memory T cells	CD3$^+$ CD45RO$^+$	6-8000-217
		CD3$^+$ CD62L$^+$ CD45RA$^-$	6-8000-216
		CD3$^+$ CD62L$^+$ CD45RO$^+$	6-8000-212
		CD45RA	6-8000-208
		CD45RO	6-8000-209
	Memory CD4$^+$ T cells	CD4$^+$ CD62L$^+$ CD45RA$^-$	6-8000-215
		CD4$^+$ CD62L$^+$ CD45RO$^+$	6-8000-211
	Memory CD8$^+$ T cells	CD8$^+$ CD62L$^+$ CD45RA$^-$	6-8000-213
		CD8$^+$ CD62L$^+$ CD45RO$^+$	6-8000-210
	Regulatory T cells	CD4$^+$ CD25$^+$	6-8000-205
		CD4$^+$ CD25$^+$ CD45RA$^+$	6-8000-214

*The kits comprise all necessary reagents.

CELL STIMULATION & EXPANSION (CD3/CD28)
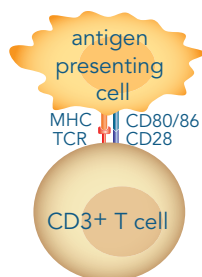
Stimulation of T cell by two stimulatory signals
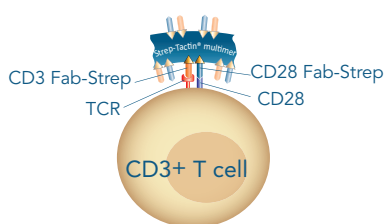
Stimulation of T cell by CD3/CD28 Streptamers for cell expansion

The *in vitro* generation of a large number of functional T cells is important for basic research as well as for therapeutic approaches. The Streptamers for T cell expansion are novel reagents for poly-clonal expansion of T cells. They are soluble protein complexes generated by multimerization of anti-CD3 and anti-CD28 Fab-Streps with a Strep-Tactin® multimer.

Streptamer® expansion technology
The Streptamers for T cell expansion are reagents for polyclonal expansion of T cells:
› Soluble protein complexes generated by multimerization of αCD3- and αCD28 Fab-Streps with a Strep-Tactin® multimer
› Completely reversible reagents, i.e. they can be removed from the cells by the addition of biotin

Key benefits
› Completely reversible reagents
› Detachment from the cells at any given point in time
› Full control about your expansion experiment
› Adjustable CD3 : CD28 ratio
› Non-magnetic and bead-free
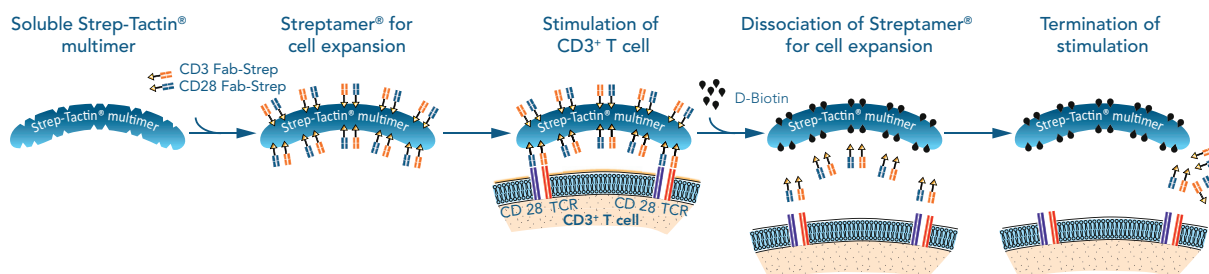
Workflow of T cells activation using Streptamers for cell expansion. After stimulation, the subsequent biotin-induced dissociation of the reagents allows an accurately defined termination of stimuli.

Streptamer® CD3/CD28 Kit for T cell expansion

Products for Stimulation and Expansion of human T cells

Cat. no.	Product description
6-8900-000	Streptamer® CD3/CD28 Kit for T cell expansion
6-8901-000	Streptamer® CD3/CD28 premix for T cell expansion

DETECTION AND ISOLATION OF ANTIGEN-SPECIFIC T CELLS WITH MHC I STREPTAMERS

Antigen-specific CD8$^+$ T cells are stained with fluorescent MHC I Streptamers (complex from fluorescence-labeled Strep-Tactin® and multimerized low-affinity MHC I-Streps) and are ready for e.g. flow cytometry. Alternatively, antigen-specific cell isolation is suitable via magnetic beads conjugated to MHC I Streptamers. The subsequent removal of label with biotin preserves the authentic properties of the cells and allows unbiased downstream applications with functional, label-free cells.

### MHC I Streptamer® consists of two components
> MHC I-Strep, which is an MHC I molecule fused with Twin-Strep-tag® and a specific antigenic peptide
> Strep-Tactin® multimers labeled with a fluorophore (PE or APC) or bound to magnetic microbeads

Key benefits
> Accurate labeling and great staining intensities due to multivalent binding
> Choose from more than 60 differenct MHC I Streptamers and corresponding peptides
> Modular products for FACS analysis and magnetic cell isolation
> Reversible reagents for full functionality of selected cells
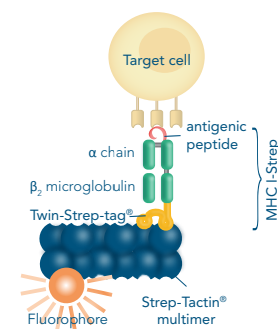
Cell staining via fluorescence labeled MHC I Streptamers
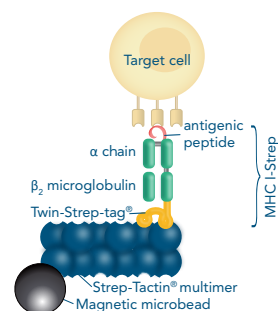
Cell isolation via magnetic beads conjugated to MHC I Streptamers

MHC I Streptamers: most relevant antigens

Species	Research field	Allele	Antigen	Sequence	Cat. No.
human	Cytomegalovirus	HLA-A*0201	CMV pp65	NLVPMVATV	6-7001-001
		HLA-B*0702	CMV pp65	TPRVTGGGAM	6-7027-001
		HLA-A*2402	CMV pp65	QYDPVAALF	6-7028-001
		HLA-A*1101	CMV pp65	GPISGHVLK	6-7050-001
		HLA-A*0101	CMV pp50	VTEHDTLLY	6-7024-001
		HLA-B*0801	CMV IE-1	QIKVRVDMV	6-7017-001
		HLA-A*0201	CMV IE-1	VLEETSVML	6-7041-001
	Tumor	HLA-A*0201	WT-1 (Wilms tumor protein)	RMFPNAPYL	6-7019-001
		HLA-A*0201	HA-1H	VLHDDLLEA	6-7018-001
		HLA-A*0201	MART 1	ELAGIGILTV	6-7007-001
		HLA-A*0201	NY-ESO-1 (cancer testis antigen)	SLLMWITQV	6-7013-001
	Epstein-Barr virus (EBV)	HLA-A*0201	EBV BMLF-1	GLCTLVAML	6-7002-001
	Influenza	HLA-A*0201	Influenza A virus M1 protein	GILGFVFTL	6-7003-001
	HIV	HLA-A*0201	HIV-1 reverse transcriptase	ILKEPVHGV	6-7005-001
mouse	Transgenic mouse OVA model	H-2 Kb	Ovalbumin	SIINFEKL	6-7015-001

Peptides of interest not listed above?
Visit our webpage: www.iba-lifesciences.com/antigen-specific-t-cell-staining-product-shop
Contact us: info@streptamer.com

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