Mouse Regulatory T Cell Staining Kit
小鼠调节性 T 细胞染色试剂盒

This package insert must be read entirely before using this product. For proper performance, use the insert provided with each individual product received.

Catalog Number

KTR201 - 25

KTR201 - 100

Optimization for mouse regulatory T cell (Treg) staining in anticoagulated blood and splenocytes.
已优化的小鼠抗凝血和脾细胞的调节性T细胞(Treg)染色。

For research use only. Not for use in diagnostic procedures.
仅用于科研，不得用于临床诊断

INTRODUCTION

The regulatory T cells (Tregs), formerly known as suppressor T cells, are a subpopulation of T cells that modulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune disease. Tregs are immunosuppressive and generally suppress or downregulate induction and proliferation of effector T cells. Regulatory T cells come in many forms with the most well-understood being those that express CD4, CD25, and FOXP3 ($CD4^+CD25^+$ regulatory T cells).

The immune system must be able to discriminate between self and non-self. When self/non-self discrimination fails, the immune system destroys cells and tissues of the body and as a result causes autoimmune diseases. Regulatory T cells actively suppress activation of the immune system and prevent pathological self-reactivity, i.e. autoimmune disease. The critical role regulatory T cells play within the immune system is evidenced by the severe autoimmune syndrome that results from a genetic deficiency in regulatory T cells (IPEX syndrome).

Mouse models have suggested that modulation of Tregs can treat autoimmune disease and cancer and can facilitate organ transplantation. Their implications for cancer are complicated. Tregs tend to be upregulated in individuals with cancer, and they seem to be recruited to the site of many tumors. Studies in both humans and animal models have implicated that high numbers of Tregs in the tumor microenvironment is indicative of a poor prognosis, and Tregs are thought to suppress tumor immunity, thus hindering the body's innate ability to control the growth of cancerous cells. Recent immunotherapy research is studying how regulation of T cells could possibly be utilized in the treatment of cancer.

LIMITATIONS OF THE PRODUCT

The product is intended for flowcytometry applications. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

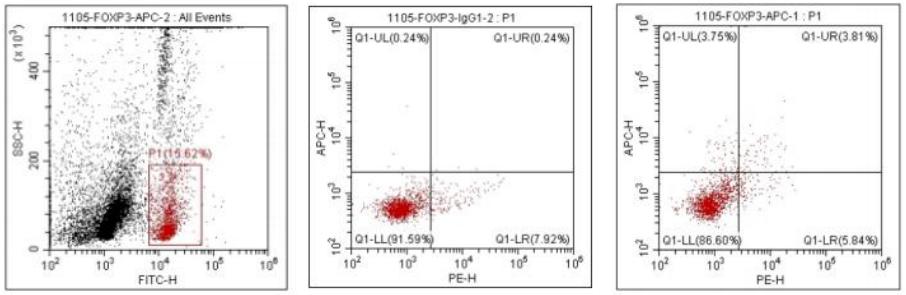
The product should not be used beyond the expiration date on the label.

Do not mix reagents with those from other lots or sources.

Related products/相关产品

Cat No.	Product Name	Size
AP101-100	Annexin V-FITC/PI 凋亡检测试剂盒	100T
CCS012	Cell cycle staining Kit	50T
CMG101	Mouse IgG1 Isotype Control, FITC	50 μg
CMG105	Mouse IgG1 Isotype Control, APC	50 μg
F0001	多聚甲醛溶液， 4%	100 ml
GAS005	FIX&PERM Kit	100T
HLSM1077	人淋巴细胞分离液	200 ml
KTH001	Human Th1/Th2/Th17 staining kit	25T/100T
KTH101	Human Th1/Th2 staining kit	25T/100T
KTH117	Human Th17 staining kit	25T/100T
KTH201	Mouse Th1/Th2 staining kit	25T/100T
KTH217	Mouse Th17 staining kit	25T/100T
KTR101	Human Regulatory T Cell staining kit	25T/100T
LSB01	Lysing solution for FACS 10×	100T
LSC01	FCM Lysing solution for BC (ready-to-use)	100T
LYS01	FCM Lysing solution (Fixative Free) 10×	100T
MLSM1092	小鼠淋巴细胞分离液	200 ml

Examples of results/结果示例



Flow cytometric analysis of Mouse Regulatory T Cell Staining Kit. *The staining pattern of FSC/CD4 (Left), CD25/Mouse IgG1 Isotype Control, APC (Middle) and CD25/FoxP3, APC (Right) on splenocytes of normal ICR mouse. Adherent cells have been excluded. Flow cytometry was performed on a Thermo Fisher Attune NxT.*

使用 Mouse Regulatory T Cell Staining Kit 进行流式检测。*正常 ICR 小鼠的脾细胞染色 FSC/CD4 (左), CD25/小鼠IgG1 同型对照，APC(中)和CD25/FoxP3, APC(右)。粘连细胞已被排除。 实验在Thermo Fisher 公司的Attune NxT 流式细胞仪上进行。*

产品介绍

调节性 T 细胞(Tregs)，曾被称为抑制性 T 细胞，是 T 细胞的亚群，可调节免疫系统、维持对自身抗原的耐受、抑制自身免疫疾病。Tregs 具有免疫抑制性， 可抑制或下调效应 T 细胞的诱导和增殖。 调节性 T 细胞有多种类型，其中研究最深的的一类表达 CD4、CD25 和 FOXP3($CD4^+CD25^+$ 调节性 T 细胞)。

免疫系统必须能区分自我和非我。当自我/非我无法识别时，免疫系统会破坏细胞和组织，引起自身免疫疾病。调节性 T 细胞积极地抑制免疫系统活化、阻止病理性的自身反应如自身免疫疾病。调节性 T 细胞遗传缺失造成的重度自身免疫综合征(IPEX 综合征)表明调节性 T 细胞在免疫系统中发挥了重要作用。

小鼠模型表明，调控 Tregs 可治疗自身免疫疾病和癌症，有助于器官移植。其在癌症中的机制比较复杂。Tregs 在癌症患者中上调，可被招募至肿瘤位点。对人和动物模型的研究显示，在肿瘤微环境中大量的 Tregs 表明不良预后。Tregs 可抑制肿瘤免疫，阻碍了机体控制癌细胞生长的能力。近来的免疫治疗研究如何利用 T 细胞的调节来治疗癌症。

产品的局限

1. 本产品用于流式细胞实验，仅用于科学研究，非诊断试剂，不能用于临床诊断。
2. 请在本产品标记的有效期内使用。
3. 本产品的试剂不能与其他批号的试剂或其他来源的试剂混合使用。

MATERIALS PROVIDED AND STORAGE

Components	Product Code	-25	-100
Anti-Mouse CD4, FITC (Clone: GK1.5)	AM00401	125 µl	500 µl
Anti-Mouse CD25, PE (Clone: PC61.5.3)	AM02502	125 µl	500 µl
Anti-Mouse FoxP3, APC (Clone: 3G3)	AM0F03	125 µl	500 µl
Anti-Mouse CD16/CD32, Purified (Clone: 2.4G2)	AM01600	60 µl	240 µl
Mouse IgG1 Isotype Control, APC	CMG103	25 µl	100 µl
Fixation/Permeabilization Concentrate (4×)	ICC01	8 ml	30 ml
Fixation/Permeabilization Diluent	ICD01	25 ml	100 ml
Permeabilization Buffer (10×)	ICB01	25 ml	100 ml
FCM Lysing Solution (Fixative Free) (10×)	LYS	8 ml	30 ml
Flow Cytometry Staining Buffer (1×)	S1001	125 ml	125 ml ×3

Note: All reagents can be stored at 2 - 8℃, and would be stable for at least 1 year when stored at recommended condition.

OTHER SUPPLIES REQUIRED

1. **Distilled water**.
2. **Rat IgG2b Isotype Control, FITC** (Isotype control for anti-mouse CD4, FITC, Maybe needed)
3. **Rat IgG1 Isotype Control, PE** (Isotype control for anti-mouse CD25, PE, Maybe needed)
4. **12 ×75 mm round bottom test tubes**.
5. **Vortexer.**
6. **Swing-out horizon centrifuge** (with rotor for 15 ml tubes).

4. 室温避光孵育 30 - 60 分钟。
5. 无需洗涤，每管加入 2 ml 1×Permeabilization Buffer。室温下 300 - 400 ×g 离心 5 分钟，弃去上清。
6. (可选)重复步骤 5。
7. 用 100 µl 1×Permeabilization Buffer 重悬沉淀。通常洗涤后残留的液体量就已足够。
8. (可选)细胞悬液中加入2 µl Anti-Mouse CD16/CD32, Purified 进行阻断，室温避光孵育 15 分钟。
9. 无需洗涤，加入 5 µl Anti-Mouse Foxp3, APC 或 5 µl Mouse IgG1 Isotype Control, APC。移液器轻柔吹打混匀（避免震荡）， 室温避光孵育至少 30 分钟。
10. 每管加入2 ml 1×Permeabilization Buffer，室温下300 - 400 ×g离心5分钟， 弃去上清。
11. (可选)重复步骤10。
12. 每管加入 500 µl 1×Flow Cytometry Staining Buffer 重悬，上机检测。

流式检测

正确设门以得到样本中的 Treg 细胞的比例。

注： 至少获取 20,000 - 30,000 个 $CD4^+$ T 细胞。 为了进行统计学差异比较，请获取足够多的细胞样本。

荧光素激发波长和发射波长

荧光素	最大激发波长(nm)	最大发射波长(nm)
FITC	495	519
R-Phycoerythrin (PE)	480;565	578
APC	650	660

4. Incubate for 30 - 60 minutes at room temperature. Protect samples from light.
5. Without washing, add 2 ml of 1×Permeabilization Buffer to each tube. Centrifuge at 300 - 400 ×g for 5 minutes at room temperature, then discard the supernatant.
6. (Optional) Repeat step 5.
7. Resuspend pellet in 100 µl of 1×Permeabilization Buffer. This is typically the residual volume after decanting.
8. (Optional) Block with Anti-Mouse CD16/CD32, Purified by adding 2 µl directly to the cells. Incubate for 15 minutes at room temperature, protect from light.
9. Without washing, add 5 µl Anti-Mouse Foxp3, APC or 5 µl Mouse IgG1 Isotype Control, APC to cells. Vortex to mix well, and incubate for at least 30 minutes at room temperature and protect samples from light.
10. Add 2 ml of 1×Permeabilization Buffer to each tube. Centrifuge at 300 - 400 ×g for 5 minutes at room temperature, then discard the supernatant.
11. (Optional) Repeat step 10.
12. Resuspend stained cells in 500 µl 1×Flow Cytometry Staining Buffer and acquire samples on a flow cytometer.

Detection by flowcytometry

Correct gating to determine the frequencies of Tregs in samples.

Note: Acquire at least 20,000 to 30,000 $CD4^+$ cells. In order to make statistically significant frequency measurements, sufficiently large sample sizes should be acquired during flow cytometric analysis.

Excitation wavelength and emission wavelength of fluorophore

Fluorophore	Ex (nm)	Em (nm)
FITC	495	519
R-Phycoerythrin (PE)	480;565	578
APC	650	660

提供的材料和贮存

组分	编号	-25	-100
抗小鼠 CD4, FITC (克隆号： GK1.5)	AM00401	125 µl	500 µl
抗小鼠 CD25, PE (克隆号： PC61.5.3)	AM02502	125 µl	500 µl
抗小鼠 FoxP3, APC (克隆号： 3G3)	AM0F03	125 µl	500 µl
抗小鼠 CD16/CD32, 纯化 (克隆号：2.4G2)	AM01600	60 µl	240 µl
小鼠 IgG1 同型对照, APC	CMG103	25 µl	100 µl
固定破膜剂浓缩液(4×)	ICC01	8 ml	30 ml
固定破膜剂稀释液	ICD01	25 ml	100 ml
破膜缓冲液(10×)	ICB01	25 ml	100 ml
红细胞裂解液(不含固定剂) (10×)	LYS	8 ml	30 ml
流式染色缓冲液(1×)	S1001	125 ml	125 ml ×3

注：所有试剂可保存于2 - 8℃， 在推荐的条件下可保存至少1年。

未提供的材料设备

1. 蒸馏水
2. 大鼠 IgG2b 同型对照, FITC (抗小鼠 CD4, FITC 的同型对照， 也许需要)
3. 大鼠 IgG1 同型对照, PE (抗小鼠 CD25, PE的同型对照，也许需要)
4. 12 ×75 mm 圆底流式管
5. 振荡器
6. 水平离心机 (配 15 ml 离心管的转子)

PROTOCOL

Sample collection

Collect appropriate amount of anticoagulant blood, store at room temperature or 2 - 8℃, and detect on the same day.

For spleen, collect and prepare as single cell suspension by using appropriate method, and remove tissue mass, detect on the same day.

Reagent preparation

Prepare 1× FCM Lysing Solution (Fixation Free) by diluting the 10× concentrate with distilled water prior to use. You will need 2 ml of 1× FCM Lysing Solution for each sample, if staining in tubes. Store excess at 2 - 8℃.

Prepare fresh Fixation/Permeabilization working solution by diluting the Fixation/Permeabilization Concentrate (1 part) with Fixation/Permeabilization Diluent (3 parts). You will need 1 ml of the Fixation/Permeabilization working solution for each sample, if staining in tubes. Do not store this buffer more than 1 day.

Prepare a 1× working solution of Permeabilization Buffer by diluting the 10× concentrate with distilled water prior to use. You will need 8.5 ml of Permeabilization Buffer for each sample, if staining in tubes. Store excess at 2 - 8℃ for up to 1 week.

Sample preparation

1. Pipet 100 µl anticoagulant blood or 1 - 10 × 10^6 splenocytes into test tubes, add 5 µl Anti-Mouse CD4, FITC and 5 µl Anti-Mouse CD25, PE to each tube. Vortex to mix well, and incubate at room temperature for 15 minutes, protect from light.
2. Add 2 ml of 1× FCM Lysing Solution work solution to each tube and pulse vortex, and incubate at room temperature for 15 minutes, protect from light. Centrifuge at 300- 400 ×g for 5 minutes at room temperature, then discard the supernatant. Add 2 ml 1× Flow Cytometry Staining Buffer, and pulse vortex. Centrifuge at 300 - 400 ×g for 5 minutes at room temperature, then discard the supernatant.
3. Add 1 ml of Fixation/Permeabilization working solution to each tube and pulse vortex.

实验步骤

样本收集和处理

收集适量抗凝血，室温或 2 - 8℃保存，并于当天进行检测。

对于脾脏组织，采集新鲜样本后通过适当的方法制备成单细胞悬液， 并去除团块，于当天进行检测。

试剂准备

用蒸馏水将 10×FCM Lysing Solution(Fixation Free)稀释为 1×工作液。在流式管中染色， 每个样本需要 2 ml 1×FCM Lysing Solution。剩余溶液保存于 2 - 8℃。

将 Fixation/Permeabilization Concentrate 和 Fixation/Permeabilization Diluent 按 1：3 比例制备成 1×工作液。在流式管中染色， 每个样本需要 1 ml。保存勿超过 1 天。

用蒸馏水将 10× Permeabilization Buffer 稀释为 1×工作液。在流式管中染色， 每个样本需要 8.5 ml。剩余溶液在 2 - 8℃最多保存 1 周。

样本制备

1. 在流式管中加入 100 µl 抗凝血或 1 - 10 ×10^6 脾细胞，加入 5 µl Anti-Mouse CD4, FITC 和 5 µl Anti-Mouse CD25, PE。涡旋震荡混匀，室温避光孵育 15 分钟。
2. 每管加入 2 ml 1× FCM Lysing Solution 工作液，涡旋震荡混匀， 室温避光孵育 15 分钟。 室温下 300 - 400 ×g 离心 5 分钟， 弃去上清。每管加入 2 ml 1×Flow Cytometry Staining Buffer，涡旋震荡混匀。室温下 300 - 400 ×g 离心 5 分钟， 弃去上清。
3. 每管加入 1 ml Fixation/Permeabilization 工作液，移液器轻柔吹打混匀（避免震荡）。