

# 人Th亚群分化方案

## 准备培养基

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1640培养基、血清(胎牛)、双抗、L-谷氨酰胺、 $\beta$ -巯基乙醇

## 培养基配置

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RPM-1640+10%胎牛血清+1mM-谷氨酰胺+55 $\mu$ M  $\beta$ -巯基乙醇+双抗

## 准备细胞

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分选出Naive CD4<sup>+</sup>T cells(CD4<sup>+</sup>CD25<sup>-</sup>CD44<sup>low</sup>CD62L<sup>high</sup>)

## 细胞刺激培养

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1. CD3包板:包板浓度2 $\mu$ g/ml(96孔板加50 $\mu$ l, 48孔板加200 $\mu$ l)  
加入上述体积后4度过夜包被, 包被后弃去孔内液体, PBS洗板2次。
2. 分选的Naive CD4<sup>+</sup>Tcells加入包被孔内。
  - a)96孔板加入1\*10<sup>5</sup>细胞(200 $\mu$ l培养体系)
  - b)48孔板加入2\*10<sup>5</sup>细胞(500 $\mu$ l培养体系)

### 3. 配置分化培养基(4μl/ml anti-human CD28+10 ng/ml Human IL2)

根据需要分化的方向额外添加以下成分:

- a) TH1, IL-12 (50 ng/ml) plus anti-human IL-4 (10 ug/ml)
- b) TH2,IL-4(50ng/ml) plus anti-human IFN-γ (10 ug/ml)
- c) TH17, IL-6 (50 ng/ml),IL-13 (10 ng/ml),IL-23 (10 ng/ml), TGF-β (10 ng/ml)plus-anti-human IFN-γ (10 ug/ml) and anti-human IL-4 (10 ug/ml)
- d) iTreg,TGF-β(10 ng/ml) plus anti-human IFN-γ (10 pg/ml) and anti-human IL-4(10 pg/ml)
- e) TH9, IL-4 (50 ng/ml), TGF-β (10 ng/ml), plus anti-human IFN-γ (10ug/ml)
- f) TH22, IL-6 (50 ng/ml),IL-23(50 ng/ml), plus anti-human IFN-γ (10 pg/ml) and anti-human IL-4 (10 pg/ml)
- g) Tfh,IL-6(50 ng/ml), IL-21 (10 ng/ml),CXCL13 (10 ng/ml).

4. 细胞培养分化3-5天,培养过程中若培养液上清变黄,更换培养基,一般建议半量换液,根据培养经验,一般是隔天换液。

5. 培养分化后鉴定(胞内染色):分化后的细胞加入20ng/ml PMA, 1μg/ml Ionomycin刺激30min后,加入10μg/ml BFA阻断,继续培养3.5h后收集细胞进行流式检测。

6. 细胞鉴定:购买相应的流式抗体及辅助试剂进行胞内染色鉴定。

#### 细胞分化所需细胞因子及抗体:

生产商	产品编号	产品名称	产品规格
PeproTech	200-01b	Recombinant Human IL-1β	2 μg/10 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	200-02	Recombinant Human IL-2	10 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	200-04	Recombinant Human IL-4	5 μg/20 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	200-06	Recombinant Human IL-6	5 μg/20 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	200-12	Recombinant Human IL-12	2 μg/10 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	200-21	Recombinant Human IL-21	2 μg/10 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	200-23	Recombinant Human IL-23	2 μg/10 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	300-47-5	Human BCA-1 (CXCL13)	5 μg/20 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
PeproTech	100-21	Human TGF-beta1-Mammalian	2 μg/10 μg/50 μg/100 μg/250 μg/500 μg/1000 μg
BioGems	05121-25	Anti-Human CD3 SAFIER Purified	100 μg/500 μg
BioGems	10311-25	Anti-Human CD28 SAFIER Purified	100 μg/500 μg
BioGems	80821-25	Anti-Human IFN-γ SAFIRE Purified	100 μg/500 μg
BioGems	81121-25	Anti-Human IL-4 SAFIRE Purified	100 μg/500 μg

生产商	产品编号	产品名称	产品规格
BioGems	05121-40	Anti-Human CD3 BG Violet 450	25 tests/100 tests
BioGems	10111-50	Anti-Human CD8a FITC	25 tests/100 tests
BioGems	80811-77	Anti-Human IFN gamma PE-Cy7	25 tests/100 tests
BioGems	81121-80	Anti-Human IL-4 APC	25 tests/100 tests
BioGems	73821-60	Anti-Human IL-17A PE	25 tests/100 tests
BioGems	44212-77-100	Mouse IgG1 Isotype Control PE-Cy7	100 µg
BioGems	42813-80-100	Rat IgG1 Isotype Control APC	100 µg
BioGems	44212-60-100	Mouse IgG1 Isotype Control PE	100 µg
BioGems	2031560-5mg	Brefeldin A	5 mg
BioGems	5608212-1mg	Ionomycin (calcium salt)	1 mg
BioGems	1652981-1mg	Phorbol 12-myristate 13-acetate	1 mg
BioGems	92110-00-150	Permeabilization Buffer (10X)	150 ml
BioGems	92160-00-160	Transcription Factor Fixation/Permeabilization	160 ml
BioGems	92550-00-50	Transcription Factor Fixation/Permeabilization Concentrate Diluent	50 ml

## 参考文献:

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