

Materials List for:

***In Vitro* Differentiation of Human CD4⁺FOXP3⁺ Induced Regulatory T Cells (iTregs) from Naïve CD4⁺ T Cells Using a TGF-β-containing Protocol**

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Materials

| Name | Company | Catalog Number | Comments |
|--|-----------------|--------------------------|---|
| All-trans retinoic acid | Sigma Aldrich | R2625-50MG | |
| anti-human Foxp3-APC clone 236A/E7 | eBioscience | 17-4777-42 | |
| anti-human CD25 microbeads | Miltenyi Biotec | 130-092-983 | |
| anti-human CD25-PE | Miltenyi Biotec | 130-091-024 | |
| anti-human CD28 antibody, LEAF Purified | Biolegend | 302914 | |
| anti-human CD3 Antibody, LEAF Purified | Biolegend | 317315 | |
| anti-human CD45RA, FITC | Miltenyi Biotec | 130-092-247 | |
| anti-human CD45RO PE clone UCHL1 | BD Biosciences | 555493 | |
| anti-human CD4-PerCP clone SK3; mIgG1 | BD Biosciences | 345770 | |
| anti-human CD8-eFluor 450 (clone OKT8), mIgG2a | eBioscience | 48-0086-42 | |
| anti-human CTLA-4 (CD152), clone BNI3, mIgG2ak, Brilliant violet 421 | BD Biosciences | 562743 | |
| anti-human IFN-g FITC clone 4S.B3; mIgG1k | eBioscience | 11-7319-81 | |
| Brefeldin A-containing solution: GolgiPlug | BD Biosciences | 555029 | |
| cDNA synthesis kit: SuperScript VILO(Reverse transcriptase) cDNA Synthesis Kit | Invitrogen | 11754-250 | |
| Density centrifugation medium: Ficoll-Paque | GE healthcare | 17-1440-03 | |
| DMSO 99.7% | Sigma Aldrich | D2650-5X5ML | |
| FBS, heat inactivated | Invitrogen | 10082-147 | |
| Fixable Viability Dye, eFluor 780 | eBioscience | 65-0865-14 or 65-0865-18 | |
| Foxp3 Staining Buffer Set | eBioscience | 00-5523-00 | Caution: contains paraformaldehyde. Can be also bought in combined kit with antibody; 77-5774-40 Anti-Human Foxp3 Staining Set APC Clone: 236A/E7 Set |

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| GlutaMAX (200 mM L-alanyl-L-glutamine) | Invitrogen | 35050-061 | |
| human naive CD4 T cell isolation kit II | Miltenyi Biotec | 130-094-131 | |
| Human serum albumin 50 g/L | Baxter | 1501057 | |
| lonomycin from Streptomyces conglobatus>98% | Sigma Aldrich | I9657-1MG | |
| MACS LS-columns | Miltenyi Biotec | 130-042-401 | |
| mouse IgG1 K Isotype Control APC Clone: P3.6.2.8.1 | eBioscience | 17-4714-42 | |
| mouse IgG1 K Isotype Control FITC 50 µg | eBioscience | 11-4714-81 | |
| mouse IgG2a isotype control, Brilliant violet 421, clone MOPC-173 | BD Biosciences | 563464 | |
| Pasteur pipet plastic, individually packed | Sarstedt | 86.1172.001 | |
| PMA Phorbol 12-myristate 13-acetate | Sigma Aldrich | P1585-1MG | |
| Rapamycin | EMD (Merck) | 553210-100UG | |
| Recombinant Human IL-2, CF | R&D | 202-IL-050/CF | |
| Recombinant Human TGF-beta 1, CF | RnD | 240-B-010/CF | |
| RNA isolation kit: RNAqueous-Micro Kit | Ambion | AM1931 | |
| RPMI 1640 Medium | Invitrogen | 72400-054 | |
| Sodium butyrate | Sigma Aldrich | B5887-250MG | |
| T cell culture medium: X-Vivo 15 medium, with gentamicin +phenolred | Lonza | 04-418Q | |
| TaqMan Gene Expression Assay, FOXP3 (Best Coverage) | Applied Biosystems | 4331182; assay ID: Hs01085834_m1 | Caution: contains paraformaldehyde |
| TaqMan Gene Expression Assay, RPL13A (Best Coverage) | Applied Biosystems | 4351370; assay ID: Hs04194366_g1 | Caution: contains paraformaldehyde |
| TaqMan Gene Expression Master mix | Applied Biosystems | 4369514 | |