

Materials List for:

# A Three-dimensional Thymic Culture System to Generate Murine Induced Pluripotent Stem Cell-derived Tumor Antigen-specific Thymic Emigrants

Raul Vizcardo<sup>\*1,2</sup>, S.M. Rafiqul Islam<sup>\*1,2</sup>, Takuya Maeda<sup>1,2</sup>, Naritaka Tamaoki<sup>1,2</sup>, Meghan L. Good<sup>1,2</sup>, Nicholas D. Klemen<sup>1,2</sup>, Marta Bosch-Marce<sup>1,2</sup>, Li Jia<sup>1</sup>, Mikhael J. Kruhlak<sup>3</sup>, Nicholas P. Restifo<sup>1,2</sup>

<sup>1</sup>Surgery Branch, National Cancer Institute, NIH

<sup>2</sup>Center for Cell-Based Therapy, National Cancer Institute, NIH

<sup>3</sup>Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, NIH

\* These authors contributed equally

Correspondence to: Raul Vizcardo at [vizcardo@nih.gov](mailto:vizcardo@nih.gov)

URL: <https://www.jove.com/video/58672>

DOI: [doi:10.3791/58672](https://doi.org/10.3791/58672)

## Materials

| Name                                                | Company                  | Catalog Number      | Comments |
|-----------------------------------------------------|--------------------------|---------------------|----------|
| <b>Chemicals, Peptides and Recombinant Proteins</b> |                          |                     |          |
| 2-deoxyguanosine                                    | Sigma-Aldrich            | 312693-72-4         |          |
| 2-Mercaptoethanol (1,000x)                          | Thermo Fisher Scientific | 21985-023           |          |
| ACK Lysing Buffer                                   | Gibco                    | A1049201            |          |
| Ascorbic acid                                       | Sigma-Aldrich            | A8960               |          |
| Blasticidin                                         | Thermo Fisher Scientific | R21001              |          |
| FBS                                                 | Gemini                   | 100-500             |          |
| Flt-3 ligand                                        | R&D Systems              | 427-FL              |          |
| GlutaMAX (100x)                                     | Thermo Fisher Scientific | 35050-061           |          |
| hgp100                                              | Genscript                | 282077-1, KVPRNQDWL |          |
| Interleukin-2                                       | R&D Systems              | 402-ML              |          |
| Interleukin-7                                       | R&D Systems              | 407-ML              |          |
| MEM Non-Essential Amino Acids Solution              | Gibco                    | 11140050            |          |
| MEM powder                                          | Gibco                    | 61100061            |          |
| Monothioglycerol                                    | Sigma-Aldrich            | M-6145              |          |
| Nucleoprotein                                       | Global Peptides          | ASNENMETM           |          |
| Penicillin/streptomycin                             | Thermo Fisher Scientific | 15140-122           |          |
| Phosphate buffered saline pH 7.4 (1x)               | Thermo Fisher Scientific | 10010-023           |          |
| Puromycin                                           | Thermo Fisher Scientific | A1113803            |          |
| RPMI 1640                                           | Gibco                    | 11875093            |          |
| Sodium Pyruvate                                     | Thermo Fisher Scientific | 11360-070           |          |
| Stem Cell Factor (SCF)                              | R&D Systems              | 455-MC              |          |
| Stemfactor LIF, Mouse Recombinant                   | STEMGENT                 | 03-0011-100         |          |
| Trypsin-EDTA (0.05%), phenol red                    | Thermo Fisher Scientific | 25300-062           |          |
| <b>Cell Culture Vessels and others</b>              |                          |                     |          |
| 10 cm dish                                          | Corning, Inc.            | 353003              |          |
| 12 mL Syringe                                       | Covidien Monoject        | 22-652-090          |          |
| 6 well plate                                        | Corning/Costar           | 3516                |          |

|                                                       |                                   |                                    |  |
|-------------------------------------------------------|-----------------------------------|------------------------------------|--|
| Cell strainer 100 µm                                  | Fisher Scientific                 | 22-363-549                         |  |
| Cell strainer 40 µm                                   | Fisher Scientific                 | 22-363-547                         |  |
| Forceps                                               | DUMONT                            | 0108-5PO                           |  |
| Lab soaker mat                                        | Versi-Dry                         | Cat. EF2175CX 74018-00             |  |
| Membrane filters ( 0.8 µm, 47diam)                    | Whatman                           | WHA7408004 ALDRICH                 |  |
| Perfecta3D Hanging Drop Plate                         | Sigma-Aldrich                     | HDP1096                            |  |
| U Bottom 96 well plate                                | Corning/Costar                    | 3799                               |  |
| <b>Experimental Cell lines</b>                        |                                   |                                    |  |
| CD3-iPSC                                              | Vizcardo et al., Cell Report 2018 | N/A                                |  |
| MEF-iPSC                                              | Vizcardo et al., Cell Report 2018 | N/A                                |  |
| Mouse Embryonic Fibroblasts (MEF)                     | ATCC                              | SCRC-1040; RRID:MGI:5007926        |  |
| OP9/N-DLL1                                            | Riken Bioresource center          | Cat# RCB2927; RRID:CVCL_B220       |  |
| Pmel-iPSC                                             | Vizcardo et al., Cell Report 2018 | N/A                                |  |
| <b>Experimental mouse models</b>                      |                                   |                                    |  |
| B6.SJL-Ptprc <sup>a</sup> Pepc <sup>b</sup> /BoyCrCrl | Charles River                     | Strain Code 564; RRID:IMSR_CRL:564 |  |
| C57BL/6N                                              | NCI/Charles River                 | N/A                                |  |
| Pmel-1 mice                                           | Overwijk et al.                   | J Exp Med 198(4):569-80            |  |
| <b>Antibodies</b>                                     |                                   |                                    |  |
| Anti-aTCR                                             | Biolegend                         | 109202; RRID:AB_313425             |  |
| Anti-CD3                                              | abcam                             | ab11089; RRID:AB_369097            |  |
| Anti-CD4                                              | BD Biosciences                    | 553730; RRID:AB_395014             |  |
| Anti-CD44                                             | BD Biosciences                    | 559250; RRID:AB_398661             |  |
| Anti-CD45.1                                           | BD Biosciences                    | 553775; RRID:AB_395043             |  |
| Anti-CD45.2                                           | BD Biosciences                    | 553772; RRID:AB_395041             |  |
| Anti-CD62L                                            | BD Biosciences                    | 560516; RRID:AB_1645257            |  |
| Anti-CD69                                             | BD Biosciences                    | 552879; RRID:AB_394508             |  |
| Anti-CD8a                                             | BD Biosciences                    | 557959; RRID:AB_396959             |  |
| Anti-CD8b                                             | BD Biosciences                    | 550798; RRID:AB_393887             |  |
| Anti-H-2Kb                                            | BD Biosciences                    | 553570; RRID:AB_394928             |  |
| Anti-IFN-g                                            | BD Biosciences                    | 557998; RRID:AB_396979             |  |
| Anti-IL-2                                             | BD Biosciences                    | 554428; RRID:AB_395386             |  |
| Anti-TCRb                                             | Thermo Fisher Scientific          | 35-5961-81; RRID:AB_469741         |  |
| Anti-TCRVb13                                          | BD Biosciences                    | 553204; RRID:AB_394706             |  |
| Anti-TNFa                                             | BD Biosciences                    | 557644; RRID:AB_396761             |  |