Feb 13, 2026

The establishment of an in vitro immunotherapy model based on MTSs

DOI
https://dx.doi.org/10.17504/protocols.io.ewov1kxnygr2/v1

Guangli Suo[1], Zhe Zhao[1], Mengdie Yang[1], Xuan Xiong[1]

[1]CAS Key Laboratory of Nano-Bio Interface, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Jiangsu, 215123, China.

MTS

Guangli Suo

DOI: https://dx.doi.org/10.17504/protocols.io.ewov1kxnygr2/v1

Protocol Citation: Guangli Suo, Zhe Zhao, Mengdie Yang, Xuan Xiong 2026. The establishment of an in vitro immunotherapy model based on MTSs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1kxnygr2/v1

Protocol status: Working
We use this protocol and it's working

Created: February 13, 2026

Last Modified: February 13, 2026

Protocol Integer ID: 243169

Keywords: immunotherapy model, characterization of human pbmc, mtss isolation, human pbmc, mt

Funders Acknowledgements:
National Natural Science Foundation of China
Grant ID: 32470814

Abstract

Isolation and characterization of human PBMCs or CD8+ T lymphocytes, followed by their co-culture with MTS in a MWC-chip.

The isolation of PBMC

1 The peripheral blood mononuclear cells (PBMCs) were isolated from a healthy donor's whole blood sample using Ficoll-Paque density gradient medium (GE Healthcare) according to the manufacturer's instructions. Briefly, the whole blood was diluted with DPBS and layered onto Ficoll-Paque medium, followed by centrifugation at 400 g for 30 minutes. After centrifugation, the mononuclear cells located at the interface were transferred to a new tube, diluted with DPBS, and pelleted at 400 g for 10 minutes. The cells were washed once with DPBS before downstream applications. Human cytotoxic T lymphocytes (CTLs) were isolated from PBMCs using the EasySep Human $CD8^+$ T Cell Enrichment Kit (STEMCELL Technologies) following the manufacturer's instructions. Briefly, PBMCs derived from BRC patients were incubated with 50 µL/mL of Enrichment Cocktail for 10 minutes at room temperature. Subsequently, the sample was incubated with magnetic particles at a concentration of 150 µL/mL for 5 minutes at room temperature. The sample was then diluted with EasySep Buffer (STEMCELL Technologies) and subjected to cell separation using an EasySep Magnet (STEMCELL Technologies). CTLs, or $CD8^+$ T cells were recovered by transferring the cell suspension into a new tube and centrifuging at 300 g for 5 minutes, and then cultured in T cell medium (STEMCELL Technologies).

The establishment of the co-culture system with MTSs and PBMC

2 To establish the co-culture system with MTSs and PBMC, the collected MTSs were seeded into a new MWC-chip, then PBMC was stained with DID and seeded in the same MWC-chip at the ratio of PBMC /
MTSs = 5 / 1. After that, 1.5 mL SG-Medium mixed with Annexin V-FITC (Vazyme) was added in the co-cultural system and maintained at 37°C in 5% CO_2 for 48 hours and imaged by Confocal (OLYMPUS). To establish the co-culture system with MTSs and CTLs ($CD8^+$ T Cell), the sorted MTSs and CTLs were mixed with the ratio of CTLs / MTSs = 5 / 1 and seeded in a new MWC-chip. Then, 1.5 mL SG-Medium mixed with Annexin V-FITC was added in the co-cultural system and maintained at 37°C in 5% CO_2 for 48 hours and imaged by Confocal (OLYMPUS). Before assessing the anti-cancer efficacy of Nivolumab (PD-1 antibody, an immune checkpoint inhibitor) on MTSs, PD-L1 expression in MTSs was assessed by staining them with a PD-L1 antibody on a MWC-chip. In the CTLs + MTSs co-culture system for immune cell potency assay, Nivolumab and annexin V-FITC were added to the medium, followed by incubation at 37°C in 5% CO2 for 48 hours. Confocal imaging (OLYMPUS) was performed to visualize the results. Control groups included MWC-chips seeded with MTSs or MTS + CTLs without Nivolumab treatment.