🌐 Peptide-MHC Dextramer Assembly

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ABSTRACT

This protocol details the assembly of peptide-MHC dextramers for use in downstream applications, including flow cytometric detection or magnetic enrichment of T cells specific for a given peptide-MHC (pMHC). Dextramer assembly of biotinylated pMHC is performed by doping onto a dextran backbone using an economic method described previously. The resulting high valency pMHC dextramers enable profiling of immune responses that may prove difficult to detect using conventional pMHC tetramers.

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Introduction

1 This protocol details the assembly of peptide-MHC dextramers for use in flow cytometric detection or magnetic enrichment of T cells specific for a given peptide-MHC (pMHC). pMHC are generated via UV-exchange and multimerized onto a dextran backbone, which can be functionalized with any fluorophore-conjugated streptavidin. This protocol is adapted from the methods originally described by Rodenko et al. (2006) and Bethune et al. (2017).

UV exchange of peptide-MHC complexes enables the exchange of any antigenic peptide of

interest in complex with a given recombinant MHC[1]. Herein, we use commercially produced biotinylated MHC monomers complexed with UV-cleavable peptides. However, recombinant protein production methods can be performed by the user to produce their own biotinylated MHC monomers[1]. UV-exchange technology affords the benefit of assembling several distinct pMHC specificities from a single batch of recombinant pMHC. Furthermore, these specificities can be assayed simultaneously in a single sample using combinatorial fluorophore barcoding[2,3]. The use of small molecule dye streptavidin conjugates (Streptavidin-AF647, Streptavidin-FITC) is not recommended.

Dextramer assembly of biotinylated pMHC is performed by doping onto a dextran backbone using an economic method described previously[4]. In essence, the doping strategy involves substoichiometric assembly of no more than 3 pMHC molecules per streptavidin-fluorophore conjugate, and subsequent doping in of dextran-biotin to saturate the remnant biotin binding site on the streptavidin. In contrast to pMHC tetramers, which only afford a valency of four pMHC complexes per tetramer molecule, pMHC dextramers afford higher-order valencies which increase the signal-to-noise ratio of detection in a manner correlated with the molecular weight of the dextran backbone[5]. Herein, we use a 500 kDa dextran-biotin backbone, which has an empirically determined degree of biotinylation of 40 molecules per backbone[6]. Using the dextran doping strategy, a valency of 120 pMHC molecules per dextran backbone is theoretically possible.

The resulting high valency pMHC dextramers enable profiling of immune responses that may otherwise be undetectable. These dextramers can be used for magnetic enrichment using anti-fluorochrome magnetic beads to isolate and culture rare, antigen-specific T cells from human or animal specimen. The use of pre-enrichment, especially from a large sample, reduces the amount of cell culture media and cytokines needed in downstream culture. Staining can be further enhanced through the use of dasatinib pre-incubation and anti-fluorochrome antibody labeling[7].

Materials

2   Materials:
  - Biotinylated MHC monomer refolded with UV-cleavable peptide
  - Antigenic peptide of interest
  - Dextran-biotin MW 500 kDa (Nanocs cat# DX500-BN-1), reconstituted in PBS
  - Fluorophore-conjugated streptavidin of choice (e.g. Streptavidin-PE, Streptavidin-APC, Streptavidin-BV785)
  - D-Biotin
  - Sodium azide
  - 96-well V-bottom polystyrene or polypropylene plate
  - RPMI + 10-20% FBS

  Equipment:
  - 365nm UV lamp

Method

3   Peptide exchange:
  1. Block the wells of a 96-well V-bottom plate with 200uL of RPMI + 10-20% FBS. Add media,

incubate briefly (a few seconds to minutes) and remove and discard all media.

2. Add MHC monomer and peptide to a final concentration of 4uM and 100-1000 ug/mL, respectively, in a well of the 96-well plate.
3. Place plate on ice with 365nm UV lamp 2-5cm above plate, or as close as possible. Cover lamp and plate with foil to protect surroundings.
4. Irradiate for 30 min to 1hr on ice. Exchange reaction efficiency is dependent on several factors, including: affinity of peptide for MHC, volume of reaction in well, and ability of reaction
5. Add streptavidin conjugate (e.g. Streptavidin-PE) to the exchange reaction to a 2.5-3:1 molar ratio of MHC:Streptavidin. Use the concentration of streptavidin component only, excluding the fluorophore, to calculate the ratio. Use the initial concentration of the MHC component, which is biotinylated at a 1:1 molar ratio of MHC:biotin when biotinylated by conventional Avitag biotinylation by BirA. Assume. Do not perform dropwise addition as the goal in this step is to assemble low order pMHC-streptavidin complexes.
6. Incubate 15 min. on ice.

Dextran doping:
1. Add dextran-biotin to a molar ratio of 20:1 streptavidin:dextran-biotin. Again, use only the streptavidin component of the conjugate to calculate molar mass. The biotin component weight of the dextran-biotin is negligible relative to the backbone (9.76 kDa biotin per 500 kDa dextran-biotin backbone, assuming 40 biotin sites per backbone).
2. Incubate 15 min. on ice
3. Block the reaction by adding D-Biotin to a final concentration of 25-30uM and sodium azide to a final concentration of 0.02% wt/vol.
4. Store at 4C and use the dextramer at 1-10ug/mL pMHC final concentration when staining cells, calculated based on initial monomer concentration. Before use, spin the dextramer at 10,000xg - 20,000xg at 4C for 1-2min to pellet aggregates, and use the supernatant to stain cells.

Key Assumptions

4. Assumptions are made in the above protocol, which may be sources of error depending on the model system being investigated (i.e. choice of peptide and MHC allotype):

- It is assumed that 100% of MHC is rescued during UV-exchange. If the peptide of interest is not sufficiently affine for cognate MHC, higher concentrations of peptide may be necessary to achieve close to 100% rescue. ELISA can be used to determine the extent of MHC stabilization when exchanged with the peptide of interest. In practice, we use TAP-deficient T2 cells expressing the MHC allele of interest to determine MHC stabilization by FACS after an overnight peptide pulse.
- The MW distribution of dextran-biotin is assumed to be normally distributed with a mean MW of 500 kDa. Without confirmation by HPLC, the possibility that lower or higher MW dextran backbones are present cannot be ruled out.

References

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