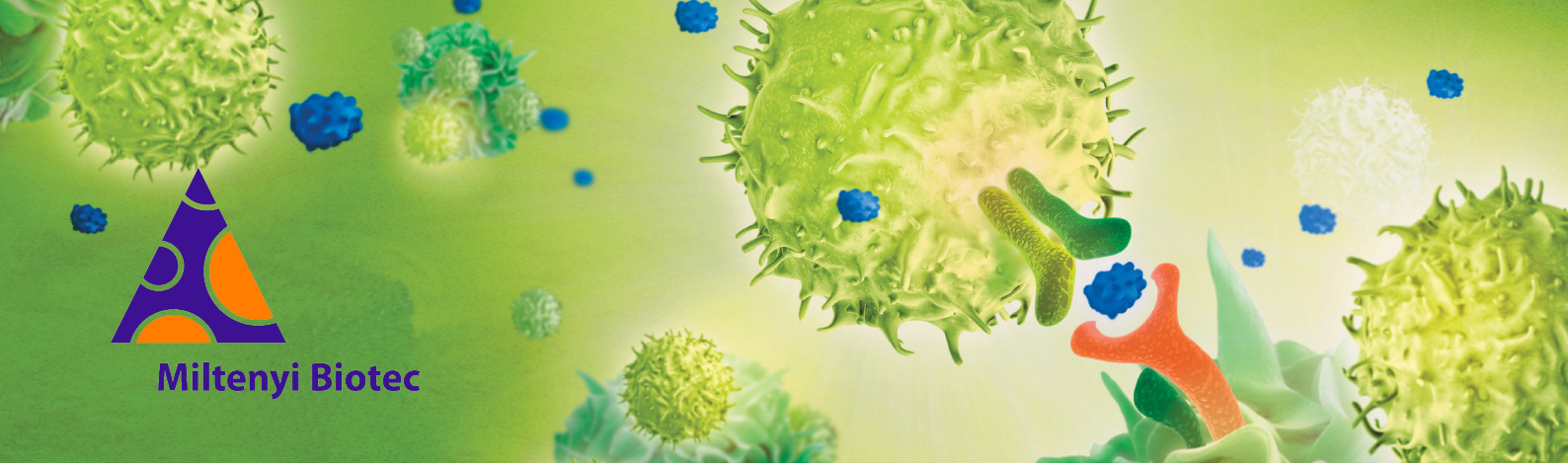


MHC MACSimer FleX Kits short instructions

Peptide Loading using MHC MACSimer FleX Kits

Introduction

This short instruction guides the user on selecting a suitable single peptide sequence for peptide loading onto MHC MACSimer FleX Kits by making a scientifically educated decision. However, it does not guarantee successful peptide loading in any given case.

Selecting a single peptide for loading onto MHC MACSimer FleX Kits

Make sure that the selected peptide is naturally restricted to match the MHC allele variant you have selected from the Miltenyi Biotec MHC MACSimer FleX Kit portfolio. There are three strategies to preselect a peptide:

- Miltenyi Biotec MACSpep Single Peptides. They are specifically designed for loading onto MHC multimers. Select from a variety of peptide sequences related to cancer, infection, or autoimmunity, and combine them with MHC MACSimer FleX Kits for maximum flexibility and optimized protocols.
- a peptide based on immunological data, such as the Immune Epitope Database (IEDB) or SYFPEITHI (T cell epitope).
- single peptides of appropriate length for the allele of interest representing a potential T cell epitope, for example, ones identified in T cell activation assays.

Peptides from the Miltenyi Biotec MACSpep Single Peptides portfolio are carefully selected from available epitope databases (e.g. IEDB, SYFPEITHI (T cell epitope)). The main specificity of the peptide given in the data sheet has been proven to bind to the respective allele. However, completeness and accuracy of this information cannot be guaranteed, especially in view of peptides with promiscuous binding specificity (e.g. HLA-DR epitopes).

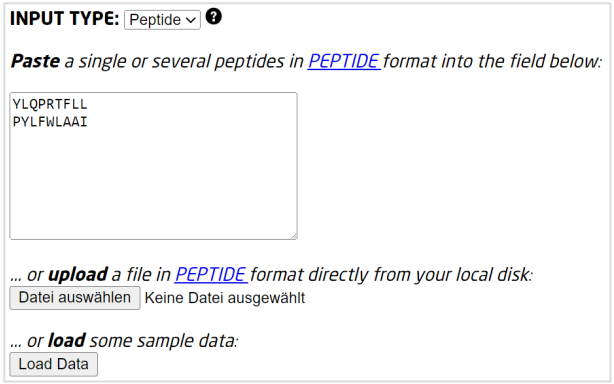
When working with peptides identified by own experiments, or peptides which are not described in the literature, start by determining the binding affinity.

Determining the binding affinity of the peptide to the MHC allotype of interest

Use an algorithmic prediction tool to determine the binding affinity of your peptide to the MHC allotype of interest. The recommended tools are NetMHCpan 4.1 for MHC I alleles and NetMHCIIpan 4.3 for MHC II alleles.

1 Set the **INPUT TYPE** to **Peptide**.

2 Add the desired peptide sequences in the text box below. Paste one line per sequence.



3 From the **SELECT SPECIES/LOCI:** dropdown list, select the MHC alleles of interest to predict the peptide binding affinity for. It is possible to select multiple alleles at once.

The most common alleles are already displayed per default.

4 Scroll to the **ADDITIONAL CONFIGURATION**.

5 Select the **Include BA predictions** checkbox.

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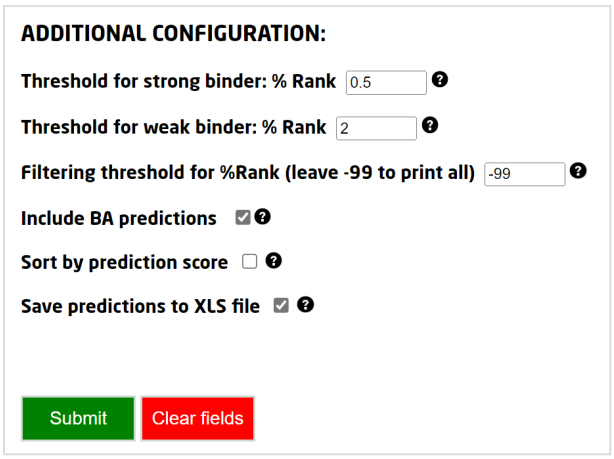
6 To download and open the results in a spreadsheet software, select the **Save predictions to XLS file** checkbox.



7 Click **Submit**. The program calculates the results.

Depending on the number of peptides and MHC alleles selected, this may take a few minutes.

Data output

The data output is listed in tables, one table row for each MHC allele.

- column **MHC** (blue box) shows the MHC allele
- column **Peptide** (blue box) shows the entered peptide sequences
- columns **%Rank_BA** and **Aff(nM)** (orange box) show the peptide binding affinities
- column **Aff(nM)** ((orange box)) shows the binding affinity of the input peptide to the specific allele in nM

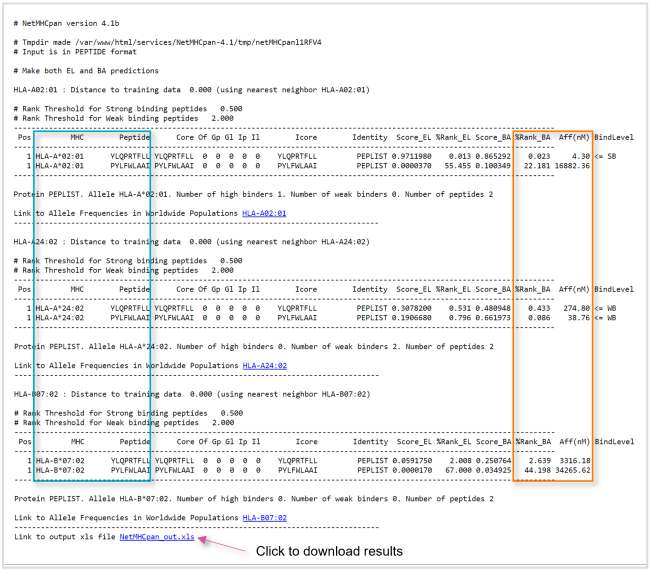


Figure 1: Data output

Predicted binding affinity interpretation

A peptide binding affinity lower than 50 nM indicates high affinity binders, while a value between 50 nM and 500 nM indicates medium affinity binders. Some epitopes may have even lower affinities, but so far no T cell epitopes with binding affinities higher than 5000 nM have been reported.

Mean absolute binding affinities are MHC allele-dependent. To compare binding affinities between different alleles, it is recommended to look at the **%Rank_BA** column. This value ranks the binding affinity in the context of a pool of random natural peptides. The lower the rank, the higher the predicted peptide-binding affinity. Ranks lower than 0,5% are usually considered strong binders, while ranks up to 2% are considered weak binders. Peptides with predicted binding affinity ranks higher than 2% are considered non-binders.

In **Figure 1**, only the first peptide, YLQPRTFLL, is predicted to bind to HLA-A*02:01. For HLA-A*24:02, both peptides are predicted to bind, but the second one, PYLFWLAAI, is predicted to have a higher affinity. Neither of the two peptides is predicted to bind to HLA-B*07:02.

See the tabs **Instructions** and **Output format** for more details on how to input data and how to interpret the output of NetMHCpan-4.1/NetMHCIIpan-4.3 (**Figure 2**).

Figure 2: Tabs of NetMHCpan tool

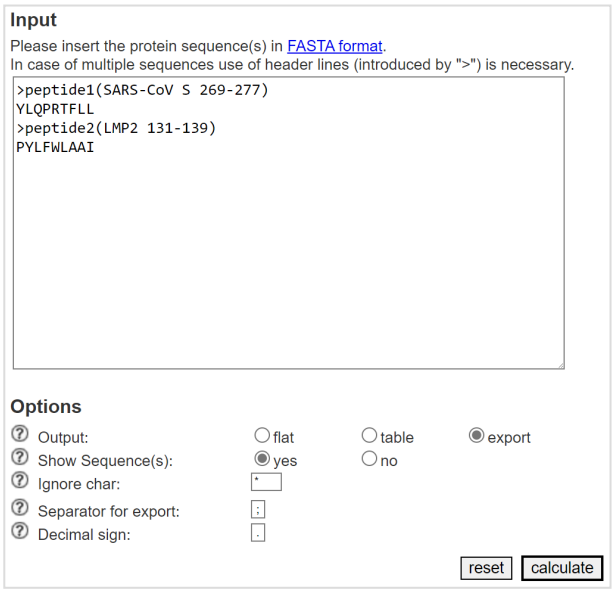
Determining the GRAVY score of the single peptide

One possible indicator for peptide-water solubility is the grand average of hydropathy (GRAVY) score, which can be calculated with a variety of web tools. If the GRAVY score of a peptide is higher than 1, a lower peptide concentration for loading might be necessary to ensure optimal loading conditions (Kyte J., Doolittle R. F., J Mol Biol, 1982).

The GRAVY score cannot serve as a sole point of reference to define the optimum peptide loading concentration.

The GRAVY score for a single peptide or a list of peptides can be determined using the GRAVY calculator.

1 In the **Input** field, enter the sequences of your single peptides of choice in FASTA format.



2 Select the desired output format. To copy and paste the results into a spreadsheet software, select the radio button **export** as **Output** in the **Options** section.

3 To view the results, click **calculate**. See **Data analysis** for details.

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Data analysis

GRAVY scores are highlighted in orange.

```
header;sequence;GRAVY
>peptide1(SARS-CoV S 269-277);YLQPRTFLL;0.28888888888889
>peptide2(LMP2 131-139);PYLFWLAAI;1.6333333333333
```

Figure 3: GRAVY scores

- Peptide 1 has a score lower than 1, thus should be soluble in aqueous buffers and should not cause issues during peptide loading.
- Peptide 2 has a score higher than 1 indicating that it is poorly soluble in aqueous buffers. It might be loaded inefficiently onto MHC MACSimer FleX Kits or even cause aggregation during peptide loading with the standard loading protocol.

Combining the results from binding affinity predictions and the GRAVY score, peptide 1 is a suitable peptide to be loaded onto MHC MACSimer A*0201 FleX Kit, human. It could also be tested for MHC MACSimer A*2402 FleX Kit, human.

For peptide 2, even though the predicted affinity for HLA-A*24:02 is high, peptide loading onto MHC MACSimer A*2402 FleX Kit, human might be challenging following the standard peptide loading protocol, because the GRAVY score suggests poor solubility of the peptide.

Because of the low predicted affinities, neither of the two peptides is suitable for loading onto MHC MACSimer B*0702 FleX Kit, human.

Recommendation for testing peptides that are not ideal for loading onto MHC MACSimer FleX Kits

If the peptide is not ideal for loading onto MHC MACSimer FleX Kits as determined with the parameters above, perform the recommended experimental optimization steps below:

- If the GRAVY score is higher than 1 with high peptide binding affinity, decrease the concentration of the peptide stock in DMSO (<10 mmol/L) and use a lower peptide concentration for MHC MACSimer FleX Kit loading. Make sure the DMSO concentration in the loading reaction stays at or below 2%. For recommended concentrations, see **Table 1.**
- If the GRAVY score is lower than 1 with low peptide binding affinity, increase the concentration of the peptide stock in DMSO (>10 mmol/L) and use a higher peptide concentration for MHC MACSimer FleX Kit loading. Make sure the DMSO concentration in the loading reaction stays at or below 2%. For recommended concentrations see **Table 1.**
- In all cases, check peptide loading reactions more carefully for signs of aggregation like clouding of the peptide stock or loading reaction, or a visible pellet after centrifugation of the peptide loading reaction.

Recommended peptide stock concentrations and required DMSO volumes

	Final peptide stock concentration (mmol/L)	Final peptide-loading concentration (µmol/L)	DMSO volume for 200 nmol peptide (µL)
Alternative concentration for peptides with low binding affinity and low GRAVY score	20	400	10
Recommended standard concentration	10	200	20
Alternative concentrations for peptides with high binding affinity, but high GRAVY score	5	100	40
	2.5	50	80
	1.25	25	160

Table 1: Recommended peptide stock concentrations and required DMSO volumes for dilution of 200 nmol peptide.

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