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Trimming of MHC Class I Ligands by ERAP Aminopeptidases

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Abstract

Studies over the last decade on characterization of the major histocompatibility complex (MHC) class I antigen presentation pathway have highlighted the importance of antigen processing, peptide transport, peptide trimming, and peptide selection as key stages for the development of optimal peptide repertoires that are presented by MHC class I molecules to cytotoxic T lymphocytes (CTLs). The study of these stages and how they are regulated, is fundamental for progress in understanding the adaptive immune system. Here we describe an in vitro assay monitoring peptide trimming by the human endoplasmic reticulum amino peptidases 1 (ERAP1) and ERAP2 (ERAPs) as a tool to characterize trimming events and gain a better understanding of the role and function of ERAPs in peptide repertoire development. Specifically, our assay allows for monitoring trimming of free but also of MHC I-bound peptides which may reflect the physiological situation best.

Keywords

Endoplasmic reticulum aminopeptidases; MHC class I molecule; Precursor peptides; Antigen processing; Mass spectrometry

1 Introduction

A key event of MHC class I maturation in the ER is the process by which antigenic peptides are selected for binding onto MHC I molecules. This process requires first that precursor peptides present in the ER be trimmed to the correct lengths of eight to ten residues in order to fit optimally within the MHC I groove. The trimming of precursor peptides is done by the ERAP aminopeptidases. That ERAPs can trim free peptides is a conceptually well accepted view of how these enzymes are thought to function in the class I pathway [1]. The idea however that ERAPs can also trim peptides that are bound (or partially bound) onto MHC I is supported by in vitro and cell based studies [2, 3], suggesting that “on MHC I” peptide trimming is likely physiologically relevant as well.

The versatility shown by ERAPs in trimming different molecular forms of precursor peptides has significant physiological implications for shaping the peptide repertoires [4]. To better understand these processes, it is necessary to have an in vitro assay whereby the consecutive removal of amino acid residues from precursor peptides by ERAPs can be probed. Here, we describe experimental procedures to generate recombinant soluble human ERAP1 and ERAP2 heterodimers (ERAP1/2) stabilized on sepharose beads [5]. We present

an in vitro assay in which bead-immobilized ERAP complexes are used to trim free and MHC I-bound peptides as monitored by mass spectrometry (MS) [6].

2 Materials

2.1 Buffers

1. Bead coupling buffer: 0.1 M NaHCO₃, pH 8.3, 0.5 M NaCl.
2. Bead blocking buffer: 0.1 M Tris-HCl, pH 8.0 or 1 M ethanolamine, pH 8.0.
3. Acidic wash buffer: 0.1 M acetic acid-sodium acetate, pH 4, 0.5 M NaCl.
4. Alkaline wash buffer: 0.1 M Tris-HCl, pH 8.0, 0.5 M NaCl.
5. Bead storage buffer: 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% NaN₃.
6. Denaturation buffer: 20 mM Tris-HCl, pH 7.5, 6 M guanidinium hydrochloride, 150 mM NaCl.
7. Dialysis buffer 1: 20 mM Tris-HCl, pH 7.5, 8 M urea, 150 mM NaCl.
8. Dialysis buffer 2: 20 mM Tris-HCl, pH 7.5, 150 mM NaCl.
9. FPLC running buffer: 20 mM Tris-HCl, pH 7.5, 150 mM NaCl.
10. Trimming buffer: 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 100 µM ZnCl₂, supplemented with 1 mM dithiothreitol immediately before use.

2.2 Common Reagents and Lab Equipment

1. Anti-ERAP1 antibody, clone 4D2 [1].
2. Anti-ERAP2 antibody, clone 3F5 [1].
3. Glycine.
4. Synthetic peptide: stock solution at 10 mg/mL in water or DMSO.
5. DTT: stock solution at 1 M.
6. HCl: solution at 1 mM HCl.
7. BaculoGold DNA (BD Pharmingen, San Jose, CA) (*see* Note 1).
8. ExCell 420 medium (Sigma-Aldrich, Saint-Quentin-Fallavier, France).
9. Grace's Insect Medium (Thermo Fisher, Waltham, MA), 1× and 2× Concentrated.
10. SF9 insect cells (Thermo Fisher, Waltham, MA).
11. Hi5 insect cells (Thermo Fisher, Waltham, MA).
12. Cellfectin (Thermo Fisher, Waltham, MA).
13. Agarose 4% (Thermo Fisher, Waltham, MA).
14. Neutral Red solution (Sigma).

15. CnBr Sepharose (GE Healthcare).
16. Laemmli sample buffer.
17. SYPRO Orange Gel Stain (Sigma-Aldrich, Saint-Quentin-Fallavier, France).
18. Leu-AMC and Arg-AMC (Bachem, Bubendorf, Switzerland).
19. PMSF.
20. Sintered glass filter.
21. Vacuum pump.
22. Syringes, 25 g.

3 Methods

3.1 Expression of Recombinant ERAP1, 2 Monomers and Heterodimers in Insect Cells

3.1.1 Baculovirus Production

1. Detach SF9 cells with ExCell 420 medium (without FBS). Count and seed 1×10^6 cells/well in a 6-well plate. Put the plate inside a humidified chamber. Leave to adhere at 27 °C for 30 min.
2. In the meantime, prepare two solutions in polypropylene tubes. Tube A: mix 3 µg DNA (ERAP1-Jun/Fos or ERAP2Fos in pAcUW51 or pVL1393 baculovirus vector (*see* Note 1) with 2 µL BaculoGold DNA and 100 µL ExCell 420 medium; and Tube B: mix 9 µL Cellfectin with 100 µL ExCell 420 medium. Combine mixtures from tubes A and B and incubate for 15–45 min at room temperature.
3. Add 800 µL ExCell 420 to the transfection mix.
4. Remove medium from the cells and replace it with the transfection medium without disturbing the cell layer.
5. Incubate overnight at 27 °C.
6. In the morning, add 2 mL fresh ExCell 420 medium containing 2.5% FBS.
7. Two days later, add 2 mL more medium containing 2.5% FBS.
8. On day 5 (2 days later), centrifuge the supernatant for 5 min at 4000 rpm (JA-14 rotor) and filter it through a 0.2 µM filter (*see* Note 2). Keep at 4 °C.

3.1.2 Plaque Assay

1. Detach SF9 cells with ExCell 420 medium (without FBS). Count and put 1×10^6 cells/well in a 6-well plate (*see* Note 3). Put plate inside a humidified chamber. Leave to adhere for 30 min.
2. Prepare dilutions of the transfection supernatant obtained in Subheading 3.1.1 ranging from $1:10^2$ (1.5 mL medium and 15 µL polyclonal virus supernatant) to $1:10^6$, in 1.5-mL reaction tubes.

3. Remove medium from cells and add the supernatant dilutions followed by incubation for 1 h at 27 °C.
4. During this hour prepare the agar: Separately boil water and 4% agarose in the microwave oven. When hot mix in a sterile manner 2 mL of water with 3.33 mL agarose in a heat-resistant 15-mL reaction tube.
5. Incubate this mixture in a water bath at 43 °C for 30 min.
6. In another 15-mL-reaction tube mix 6.67 mL 2× concentrated Grace's Medium and 1.33 mL FBS. Incubate at 43 °C for 30 min.
7. After 30 min, combine the solutions from the two reaction tubes and leave at 43 °C until the end of the 60-min infection time.
8. When the infection time is over, remove viral supernatant and add very slowly 2 mL of agarose medium from the side of each well.
9. After polymerization, incubate at 27 °C for 5 days.
10. After 5 days, prepare another agarose solution in Grace's medium following **steps 3 and 4**.
11. Just before adding the warm agarose solution to the wells, add Neutral red to it (Sigma stock diluted 1:66) (*see* Note 4). Incubate at 27 °C. After 24 h, the lysis plaques are visible as white dots in the red cell layer (*see* Note 5).
12. Circle about ten well-separated lysis plaques per dilution construct with a marker on the bottom of the plate.

3.1.3 Amplification of a Lysis Plaque

1. Detach SF9 cells with ExCell 420 medium (without FBS). Count and seed 1.5×10^5 cells/well in 150 µL medium with 2.5% FBS in a 96-well plate. Put the plate inside a humidified chamber. Leave to adhere at 27 °C for 30 min.
2. Pick a virus isolate using a pipette with a 1000 µL filter tip by pushing it through the agar to the bottom of the plate where a lysis plaque has been marked (*see* Note 6).
3. Repeat **step 2** for the desired number of viral plaques (*see* Note 7).
4. Add the small agar punch-out in one well of the 96-well plate seeded with Sf9 cells. Incubate at 27 °C for 3 days.
5. Recover 150 µL supernatant from each well and transfer to a labeled 1.5-mL reaction tubes. Set aside another 10 µL in correspondingly labeled tubes to test virus clones by PCR (*see* Note 8).
6. With 50 µL from the first monoclonal supernatant, you can infect 2×10^6 cells to obtain the first 5 mL of infectious supernatant for a most likely monoclonal virus.

3.1.4 Initial Amplification of a Baculovirus: First 5 mL of High-Titer Viral Supernatant (P1 Passage)

1. Plate 2×10^6 SF9 cells in a T-25 flask and let adhere for 30 min.
2. Mix 50 μ L monoclonal viral supernatant with 500 μ L ExCell medium without FBS.
3. Withdraw the culture medium from the cells.
4. Add the virus dilution to the cells and incubate at 27 °C for 1 h. Gently agitate the flask every 20 min to avoid drying of the cell layer (*see* Note 9).
5. Remove the virus solution and add 5 mL of ExCell medium.
6. Collect the supernatant after 4 days and store it at 4 °C (*see* Note 10).

3.1.5 Amplification of Baculovirus: 20 mL of High-Titer Viral Supernatant (P2 Passage)

1. With 150 μ L from the P1 supernatant, infect 20×10^6 cells in a T-175 flask to obtain 20 mL of monoclonal virus: Mix 150 μ L P1 supernatant with 2.85 mL ExCell medium without FBS and infect the cells for 1 h (swirling gently every 20 min).
2. Remove the virus solution and add 20 mL medium.
3. Monitor signs of Sf9 cell infection starting on day 2 of culture. We usually stop the cultures when 30–50% of cells have been killed or look moribund (loss of halo, granular appearance) which generally occurs after 3–4 days. Collect 20 mL supernatant and store it at 4 °C (*see* Note 11).

3.1.6 ERAP Monomer and Heterodimer Expression

1. Grow Hi5 cells to confluency (60×10^6 Hi5 cells for a T-175 flask) (*see* Note 12).
2. Remove the culture medium.
3. Slowly add virus-containing medium (3 mL for T-175 flask) without disturbing the cell layer. It is best to release the medium against the sides of the flask since insect cells are easily detached from plastic. For expression of monomers use 1.5 mL virus stock plus 1.5 mL medium, for expression of ERAP heterodimers use 1.5 mL ERAP1Jun virus stock plus 1.5 mL ERAP2Fos virus stock.
4. Incubate at 27 °C for 1 h, gently swirling the flask every 20 min to cover all cells.
5. After 1 h, remove the virus solution and add 15 mL medium.
6. Incubate at 27 °C between 48 and 72 h (*see* Note 13).
7. Harvest ERAP-containing supernatant, centrifuge for 10 min at 4000 rpm to spin down cells and debris and recover the supernatant.

8. Add PMSF at 0.1 mM to supernatant (*see* Note 14).

3.2 Purification of ERAP Monomers and ERAP1/2 Heterodimers via Immuno-precipitation

3.2.1 Covalent Coupling of Anti-ERAP Antibodies to CnBR Sepharose (See Note 15)

1. Five to ten milligrams of purified antibody (anti-ERAP2 clone 3F5 or anti-ERAP1 clone 4D2) are required for 1 g of lyophilized Sepharose powder, corresponding to approximately 3.5 mL of swollen Sepharose. In addition, glycine-coupled beads are prepared for preclearing of the samples following the same protocol.
2. Dialyze the antibody to be coupled in the coupling buffer (*see* Note 16). Glycine powder can be directly dissolved in the coupling buffer. Use about 5 mL coupling buffer/g lyophilized powder to be coupled (*see* Note 17).
3. Weigh out the required amount of Sepharose powder and suspend it in 1 mM HCl. The Sepharose swells immediately and should now be washed for 15 min with 1 mM HCl on a sintered glass filter (porosity G3) connected to a vacuum pump. Use approximately 200 mL of 1 mM HCl per gram freeze-dried powder, added in several aliquots.
4. Resuspend the Sepharose in 15 mL of coupling buffer and transfer the slurry into a 15-mL tube.
5. Centrifuge for 5 min at 2000 rpm (JA-14 rotor) in a tabletop centrifuge (*see* Note 18) and aspirate the supernatant.
6. Add the coupling buffer containing the antibody to the Sepharose.
7. Rotate the mixture end-over-end at 4 °C for 1 h at room temperature or overnight (*see* Notes 17 and 19).
8. Wash the slurry with at least five bead volumes of coupling buffer on a sintered glass to remove unbound antibody.
9. Resuspend the sepharose in the blocking buffer to block any remaining active groups and incubate for 2 h.
10. Perform three wash cycles with wash buffers of alternating pH: acidic wash buffer (0.1 M acetic acid–sodium acetate, pH 4.0 containing 0.5 M NaCl) and alkaline wash (*see* Note 20).
11. Prepare a slurry with storage buffer in a ratio of 20% settled medium to 80% buffer in an appropriate tube. Keep the antibody-coupled beads at 4 °C. Do not freeze.

3.2.2 Immuno-precipitation of ERAP Monomers and ERAP1/2 Heterodimers

1. Transfer a slurry volume corresponding to 30 µL net volume of glycine–sepharose beads into a 50-mL tube.

2. Add 5 mL of storage buffer, centrifuge at 2000 rpm for 5 min and aspirate the supernatant with a 25 g needle without disturbing the visible bead pellet.
3. Add the dimer-containing supernatant and leave them at least for 1 h at 4 °C on a turning wheel (*see* Note 21).
4. Centrifuge the preclearing mix at 2000 rpm for 5 min and recover the precleared supernatant.
5. Prepare separate 50-mL tubes for the immunoprecipitation: use 20 µL packed 3F5 Sepharose for purification of dimers or ERAP2 monomers (*see* Note 22), and 4D2 Sepharose for ERAP1 monomers. Wash beads once as in **step 2**.
6. Add the precleared supernatants to the beads.
7. Rotate overnight at 4 °C on a turning wheel.
8. Centrifuge the tubes at 2000 rpm for 5 min and discard the supernatant.
9. Wash beads three times with storage buffer.
10. After the last centrifugation, resuspend the pellet in 600 µL storage buffer.
11. Immediately process bead-bound ERAP1/2 enzymes: Use 200 µL of the suspension for quantification: Resuspend bead-associated peptidases in Laemmli sample buffer and separate via SDS-PAGE under denaturing conditions. Determine protein concentration relative to a BSA standard by staining with SYPRO Orange gel stain or a comparatively sensitive protein stain with a broad linear detection range following the manufacturer's instructions.
12. Immediately remove the remaining liquid from the remaining 400 µL of beads, add 13 µL of glycerol (i.e., a volume equal to packed beads) and store at –80 °C.
13. Test the enzymatic activity of each batch of ERAP1/2 beads by fluorometric monitoring of Leu-AMC and Arg-AMC (400 µM) hydrolysis, using excitation at 350 nm and measuring emission at 460 nm.

3.3 Preparation of Peptide-Deficient MHC Class I Molecules

1. Peptide-deficient MHC class I molecules are prepared from the denaturation of peptide-filled MHC class I molecules after removal of the bound peptide [7].
2. Incubate peptide-filled MHC class I molecules in the denaturation buffer for 4 hours at room temperature (*see* Note 23).
3. Transfer the mixture containing denatured peptide-filled MHC class I molecules to a 10 kDa spin column and wash extensively with the denaturation buffer to dilute away the released peptide. Collect the retentate containing the denatured class I heavy chain and β_2m subunits and dilute it with the denaturation buffer to 0.1 mg/mL protein (based on the starting concentration of peptide-filled MHC class I molecules).

4. Dialyze the mixture in 500 mL of Buffer 1 using a 6000–8000 MWCO membrane at 15 °C for 12 h. Continue dialysis for an additional 12 h with fresh 500 mL of Buffer 1.
5. Add 30% molar excess of folded β_2m (relative to the starting concentration of peptide-filled MHC class I molecules) (*see Note 24*) to the dialysis bag. Dialyze the solution in 1 L of Buffer 2 at 4 °C for 24 h. Continue dialysis for an additional 18 h with fresh 1 L of Buffer 2.
6. Add glycerol (to 15%) to the dialyzed solution (*see Note 25*), mix well, and concentrate the mixture to ~1 mL.
7. Purify the crude peptide-deficient MHC class I molecules on a gel filtration column in the FPLC running buffer, collect the corresponding fraction (*see Note 26*) and supplement it immediately with glycerol (to 15%). Concentrate the combined fractions (*see Note 27*) and store the purified protein at –80 °C. Determine the activity of peptide-deficient MHC class I molecules (*see Note 28*).

3.4 Loading of Peptides Onto Peptide-Deficient MHC Class I Molecules

This peptide binding strategy applies to 9mer as well as N-terminally extended peptides.

1. Incubate peptide-deficient molecules (*see Note 29*) with a molar excess of purified peptides (*see Note 30*) in the FPLC buffer on ice for 1 h.
2. Wash extensively the mixture containing MHC I–peptide complex in a mini-spin column (10 kDa MWCO) with the FPLC buffer to remove excess of free peptides.
3. Collect the retentate containing MHC I–peptide complex and determine the concentration (*see Note 31*) by Edelhoch's method [8]. Analyze the MHC I–peptide complex by MS (*see Note 32*) to confirm that the peptide is present.
4. The MHC I–peptide complexes generated in this manner were used in the ERAP1/2 trimming assay.

3.5 Trimming Assay Using ERAP1/2 Beads

1. Standardize each new batch of ERAP1/2 beads by incubating 3 μ L of the slurry with free 15mer peptide (RA)₃ALRSRYWAI (1.25 μ g) in the trimming buffer at 37 °C for 1 h 20 min (total reaction volume is 20 μ L) (*see Note 33*).
2. Quench the mixture with formic acid (to 1%) to inhibit the enzymes, and spin-down for 3 min in a microcentrifuge (14,000 $\times$ g). Collect the supernatant.
3. Analyze the supernatant by MS (*see Note 32*), a typical MS spectrum shows a series of peptide peaks each of which corresponds to a trimmed fragment of the 15mer (Fig. 1) (*see Note 34*).
4. Next, incubate a precursor peptide (1.25 μ g), in a free or MHC I-bound form, with 3–5 μ L of the calibrated ERAP1/2 beads (*see Note 35*) in the trimming buffer at 37 °C (total reaction volume is 20 μ L). At various times (*see Note 36*),

spin down the assay mixture for 3 min in a microcentrifuge ($14,000 \times g$). Take an aliquot (5 μ L) from the supernatant (*see* Note 37) and quench it with formic acid (to 1%). Keep the aliquots in -80°C until MS analysis (*see* Note 38).

5. The trimming assay should be repeated two to four times using different batches of calibrated ERAP1/2 beads.

4 Notes

1. The BaculoGold DNA has been discontinued. Possible alternatives that work with the same baculovirus vectors are ProEasy or ProGreen (AB Vector, San Diego, CA).
2. This is the first polyclonal viral supernatant. It might contain wild-type baculovirus and is therefore not suitable for production of high-titer viral supernatant for protein expression. A plaque assay is used to isolate recombinant virus clones containing the transfer vector.
3. The cell number used for seeding is crucial for the success of the assay because the lysis plaques will only be detectable on a confluent monolayer of cells. As the cells continue growing during the six days of the assay under the agar and the growth kinetics might vary from culture to culture it is difficult to determine the optimal cell number for seeding. However, in our hands, seeding one million of cells gave satisfying results in most plaque assays.
4. Neutral red only stains live cells. The plaques of cells lysed by virus will therefore appear white.
5. Although the plaques can be seen with bare eyes, observing them under a cell culture light microscope will help to distinguish lysis plaques (dead cells) from holes in the cell monolayer.
6. It is essential to pick only well separated plaques in order to isolate and amplify monoclonal viruses.
7. Ten clones are usually largely sufficient because the proportion of “empty” (wild-type) viruses is usually very low.
8. The screening PCR is required to validate positive recombinant clones. It can be performed with any primers binding to the introduced gene.
9. Swirling is critical to keep all cells covered with the small volume of infection medium.
10. The P1 high-titer supernatants are stable for many years. The titer will drop over time but it is usually possible to amplify it to obtain a new P2 high titer supernatant. The P1 can also be used to perform a new plaque assay in order to reamplify or reselect monoclonal virus.
11. This high-titer supernatant will be used for infections to express the recombinant proteins.

12. Hi5 insect cells are recommended because they secrete higher amounts of recombinant protein than Sf9. They can also grow well in serum-free medium. The absence of serum proteases is beneficial for the stability of the expressed and secreted ERAP proteins and facilitates their purification.
13. The optimal expression time should be determined individually and can slightly change from culture to culture.
14. ERAP enzymatic activity is not inhibited by PMSF. Do not add commercial mixes of protease inhibitors as these might efficiently and irreversibly inhibit ERAP activity.
15. Covalently coupled beads save time, work and reagents when series of immunoprecipitations are to be performed. Also, as the covalently coupled antibody is quite resistant to the acid elution, antibody contamination in the protein quantification gel will be absent or very limited.
16. The coupling reaction proceeds most efficiently in the pH range 8–10 where the amino groups on the ligand are predominantly in the unprotonated form. A buffer at pH 8.3 is most frequently used for coupling proteins. To minimize protein-protein adsorption and the formation of protein aggregates, it is recommended to have a high salt content, 0.5 M NaCl, in the coupling buffer.
17. The initial OD280 of the ligand in coupling buffer as well as the post-coupling supernatant can be measured with a spectrophotometer in order to control the coupling efficiency. A 1 mg/mL antibody solution gives an absorption of 1.4 at 280 nm. After successful coupling the OD should have drastically decreased compared to the starting solution. The coupling efficiency can be calculated as the amount of ligand in the solution divided by the starting amount before coupling. Typical coupling efficiency is above 90%.
18. High speed centrifugation might damage beads.
19. Do not use magnetic stirrers as the Sepharose can be fragmented.
20. This procedure helps to remove any remaining ionically bound ligand from the resin.
21. This preclearing step will remove any proteins non-specifically binding to the Sepharose.
22. We have shown that ERAPs precipitated from a ERAP1–2-co-infected supernatant via 3F5 are exclusively heterodimers because all ERAP2 molecules are bound to ERAP1 under these conditions [5].
23. The concentration of MHC class I molecules in the denaturation buffer should be ~10–20 mg/mL.
24. A 30% molar excess of folded β_2m is added to promote the correct pairing of class I heavy chain with β_2m subunits.

25. Glycerol helps to stabilize the intrinsically unstable peptide-deficient MHC class I molecules.
26. MHC I-peptide complexes elute from a calibrated gel filtration column at ~44 kDa.
27. Stock solutions of purified peptide-deficient MHC class I molecules should be less than 10 mg/mL. The protein concentration can be determined by Edelhoch's method [8].
28. It is recommended that the activity of peptide-deficient MHC class I molecules be determined quantitatively as described in [9] to assess the number of peptide-deficient molecules in solution that are capable of effectively binding peptides.
29. The concentration of peptide-deficient MHC class I molecules in the FPLC buffer should be ~2.5 mg/mL.
30. Synthetic peptides should be purified for this experiment. Many peptides restricted to MHC class I molecules are highly hydrophobic and DMSO can be used as the dissolving solvent. The final concentration of DMSO in the reaction mixture should be less than ~5%.
31. The stock solutions of MHC I-peptide complexes should be ~10 mg/mL.
32. MALDI-TOF or electrospray MS analysis.
33. (RA)₃ALRSRYWAI serves as a control peptide and is used to ensure that the trimming assay is reproducible such that the results from trimming of different starting precursors can be normalized.
34. Depending on the intensity of the peak corresponding to the starting 15mer (RA)₃ALRSRYWAI as well as the overall extent of trimming, the amounts of ERAP1/2 beads can be adjusted slightly so the trimming of (RA)₃ALRSRYWAI gives consistent results for each new batch of beads.
35. The exact amounts of beads to use is determined in the calibration step using free (RA)₃ALRSRYWAI.
36. Note that free peptides are trimmed significantly faster than MHC I-bound peptides [6]. Therefore, the times at which samples are collected should be determined considering the overall goal of the experiment in preliminary experiments.
37. In some experiments, when the incubation times are long, it can be necessary to supplement the reaction mixtures with calibrated ERAP1/2 beads (3–5 µL).
38. A typical MS spectrum is shown in ref. 6.

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Fig. 1.

N-terminal trimming of free and HLA-B*0801-bound (RA)₃ALRSRYWAI by ERAP1/2. Free (RA)₃ALRSRYWAI 15mer was incubated with ERAP1/2 beads at 37 °C. An aliquot was taken from the mixture after 1 h 20 min and analyzed by MALDI-TOF MS. The starting precursor (RA)₃ALRSRYWAI peptide and its fragments are identified