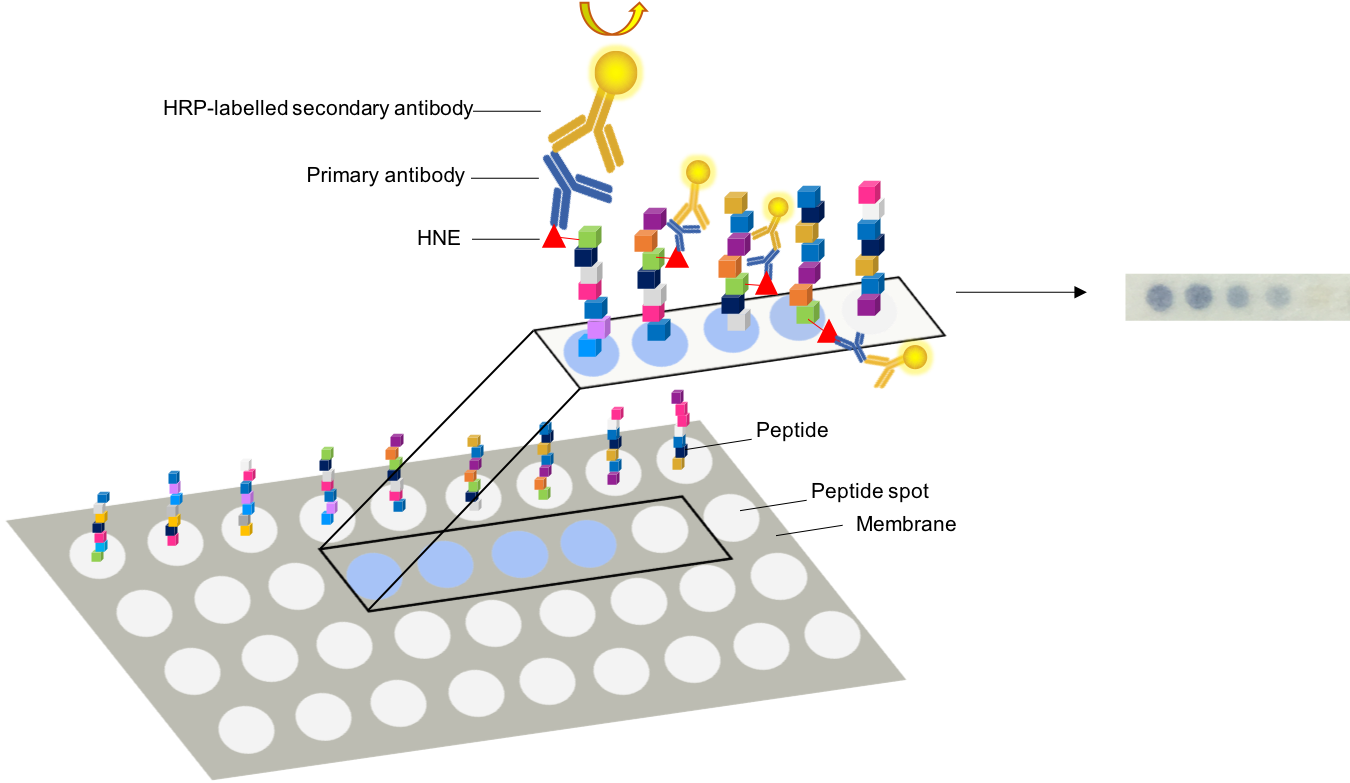
Epitope mapping and characterization of 4-hydroxy-2-nonenal modified-human serum albumin using two different polyclonal antibodies

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Free Radical Biology and Medicine

Campos-Pinto et al. Supplementary data



**Supplementary Fig. 1.** Schematic representation of the peptide array. The membrane is firstly incubated with HNE and subsequently with the primary and the HRP-labelled secondary antibody. After incubation with the substrate, the peptide spots to which the antibody is bound develop a blue colour.

**Supplementary Fig. 2.** Screening of the antibodies present in the pre-immune bleed and in four post-immunization bleeds collected from a sheep immunized with HNE-modified HSA. The binding to HNE modified Ovalbumin (OVA) was accessed by ELISA assay.

A screenshot of a cell phone

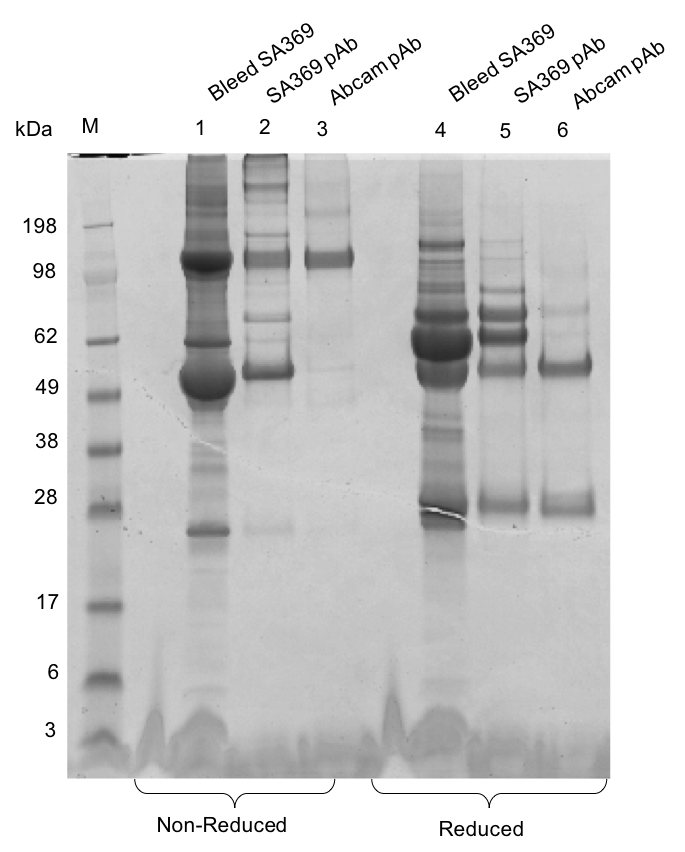
Description automatically generated

**Supplementary Fig. 3.** SA369 anti-HSA-HNE pAb enriched pAb purification chromatograms from affinity purification using an HSA-HNE-coupled resin to recover the mixture of anti-HA and anti-HSA-HNE IgGs (A) and an HSA-coupled resin for depletion of anti-HSA antibodies (B). The bound pooled fraction of the antibodies obtained in (A) (circled in red) were loading into the second column, where the enriched pooled fraction of anti-HAS-HNE was collected from the unbound fraction (circled in red). Binding assessment of the enriched pAbs to HSA, HSA-HNE, OVA and OVA-HNE by direct ELISA was performed by immobilizing the antigens at 1.25 μg/mL and testing with the antibody con.centrations indicated on the graph. Average of 3 technical replicate ± SEM.

**A picture containing text

Description automatically generated**

**Supplementary Fig. 4.** Chromatogram obtained from the purification of the IgGs from the crude sheep sera (SA369) with a protein G resin (A) and from the enrichment of anti-HNE antibodies using a KLH-HNE conjugated resin (B).



**Supplementary Figure 5.** Purification of SA369 pAbs from the sheep bleed SA369 using a KLH-HNE covalently bound to a sepharose resin. The collected pooled fractions contain still a trace of serum albumin as observed by a band corresponding to the weight of 65 kDa and some other immunoglobulins of higher molecular weight, possibly IgM. Samples in lanes 1, 2 and 3 were run under non-reducing conditions, while samples in lanes 4, 5 and 6 were run under reducing conditions. ﻿In lanes 5 and 6 bands corresponding to the weight of the heavy (50 kDa) and light chains (25 kDa) of the pAbs are seen. Proteins were separated by SDS- PAGE and stained by Coomassie blue.

**Supplementary Figure 6.** ELISA assay comparing the binding of the anti-HNE Abcam and the Anti-HSA-HNE SA369 antibodies to recombinant HSA expressed in *Saccharomyces cerevisiae* and standard HSA (the same HSA molecule used for immunization).

**Supplementary Table 1.**  List of the human serum albumin (UniProt ID: P02768) peptides synthesized on the arrays. The sequence includes both the signal peptide (1-18) and pro-peptide (19-24) of HSA.

|  |  |  |  |
| --- | --- | --- | --- |
| **Positional Reference** | **Peptide sequence** | **Positional Reference** | **Peptide sequence** |
| A1 | Blank | B26 | G-E-M-A-D-C-C-A-K-Q-E-P-E-R-N |
| A2 | M-K-W-V-T-F-I-S-L-L-F-L-F-S-S | B27 | M-A-D-C-C-A-K-Q-E-P-E-R-N-E-C |
| A3 | W-V-T-F-I-S-L-L-F-L-F-S-S-A-Y | B28 | D-C-C-A-K-Q-E-P-E-R-N-E-C-F-L |
| A4 | T-F-I-S-L-L-F-L-F-S-S-A-Y-S-R | B29 | C-A-K-Q-E-P-E-R-N-E-C-F-L-Q-H |
| A5 | I-S-L-L-F-L-F-S-S-A-Y-S-R-G-V | B30 | K-Q-E-P-E-R-N-E-C-F-L-Q-H-K-D |
| A6 | L-L-F-L-F-S-S-A-Y-S-R-G-V-F-R | C1 | E-P-E-R-N-E-C-F-L-Q-H-K-D-D-N |
| A7 | F-L-F-S-S-A-Y-S-R-G-V-F-R-R-D | C2 | E-R-N-E-C-F-L-Q-H-K-D-D-N-P-N |
| A8 | F-S-S-A-Y-S-R-G-V-F-R-R-D-A-H | C3 | N-E-C-F-L-Q-H-K-D-D-N-P-N-L-P |
| A9 | S-A-Y-S-R-G-V-F-R-R-D-A-H-K-S | C4 | C-F-L-Q-H-K-D-D-N-P-N-L-P-R-L |
| A10 | Y-S-R-G-V-F-R-R-D-A-H-K-S-E-V | C5 | L-Q-H-K-D-D-N-P-N-L-P-R-L-V-R |
| A11 | R-G-V-F-R-R-D-A-H-K-S-E-V-A-H | C6 | H-K-D-D-N-P-N-L-P-R-L-V-R-P-E |
| A12 | V-F-R-R-D-A-H-K-S-E-V-A-H-R-F | C7 | D-D-N-P-N-L-P-R-L-V-R-P-E-V-D |
| A13 | R-R-D-A-H-K-S-E-V-A-H-R-F-K-D | C8 | N-P-N-L-P-R-L-V-R-P-E-V-D-V-M |
| A14 | D-A-H-K-S-E-V-A-H-R-F-K-D-L-G | C9 | N-L-P-R-L-V-R-P-E-V-D-V-M-C-T |
| A15 | H-K-S-E-V-A-H-R-F-K-D-L-G-E-E | C10 | P-R-L-V-R-P-E-V-D-V-M-C-T-A-F |
| A16 | S-E-V-A-H-R-F-K-D-L-G-E-E-N-F | C11 | L-V-R-P-E-V-D-V-M-C-T-A-F-H-D |
| A17 | V-A-H-R-F-K-D-L-G-E-E-N-F-K-A | C12 | R-P-E-V-D-V-M-C-T-A-F-H-D-N-E |
| A18 | H-R-F-K-D-L-G-E-E-N-F-K-A-L-V | C13 | E-V-D-V-M-C-T-A-F-H-D-N-E-E-T |
| A19 | F-K-D-L-G-E-E-N-F-K-A-L-V-L-I | C14 | D-V-M-C-T-A-F-H-D-N-E-E-T-F-L |
| A20 | D-L-G-E-E-N-F-K-A-L-V-L-I-A-F | C15 | M-C-T-A-F-H-D-N-E-E-T-F-L-K-K |
| A21 | G-E-E-N-F-K-A-L-V-L-I-A-F-A-Q | C16 | T-A-F-H-D-N-E-E-T-F-L-K-K-Y-L |
| A22 | E-N-F-K-A-L-V-L-I-A-F-A-Q-Y-L | C17 | F-H-D-N-E-E-T-F-L-K-K-Y-L-Y-E |
| A23 | F-K-A-L-V-L-I-A-F-A-Q-Y-L-Q-Q | C18 | D-N-E-E-T-F-L-K-K-Y-L-Y-E-I-A |
| A24 | A-L-V-L-I-A-F-A-Q-Y-L-Q-Q-C-P | C19 | E-E-T-F-L-K-K-Y-L-Y-E-I-A-R-R |
| A25 | V-L-I-A-F-A-Q-Y-L-Q-Q-C-P-F-E | C20 | T-F-L-K-K-Y-L-Y-E-I-A-R-R-H-P |
| A26 | I-A-F-A-Q-Y-L-Q-Q-C-P-F-E-D-H | C21 | L-K-K-Y-L-Y-E-I-A-R-R-H-P-Y-F |
| A27 | F-A-Q-Y-L-Q-Q-C-P-F-E-D-H-V-K | C22 | K-Y-L-Y-E-I-A-R-R-H-P-Y-F-Y-A |
| A28 | Q-Y-L-Q-Q-C-P-F-E-D-H-V-K-L-V | C23 | L-Y-E-I-A-R-R-H-P-Y-F-Y-A-P-E |
| A29 | L-Q-Q-C-P-F-E-D-H-V-K-L-V-N-E | C24 | E-I-A-R-R-H-P-Y-F-Y-A-P-E-L-L |
| A30 | Q-C-P-F-E-D-H-V-K-L-V-N-E-V-T | C25 | A-R-R-H-P-Y-F-Y-A-P-E-L-L-F-F |
| B1 | P-F-E-D-H-V-K-L-V-N-E-V-T-E-F | C26 | R-H-P-Y-F-Y-A-P-E-L-L-F-F-A-K |
| B2 | E-D-H-V-K-L-V-N-E-V-T-E-F-A-K | C27 | P-Y-F-Y-A-P-E-L-L-F-F-A-K-R-Y |
| B3 | H-V-K-L-V-N-E-V-T-E-F-A-K-T-C | C28 | F-Y-A-P-E-L-L-F-F-A-K-R-Y-K-A |
| B4 | K-L-V-N-E-V-T-E-F-A-K-T-C-V-A | C29 | A-P-E-L-L-F-F-A-K-R-Y-K-A-A-F |
| B5 | V-N-E-V-T-E-F-A-K-T-C-V-A-D-E | C30 | E-L-L-F-F-A-K-R-Y-K-A-A-F-T-E |
| B6 | E-V-T-E-F-A-K-T-C-V-A-D-E-S-A | D1 | L-F-F-A-K-R-Y-K-A-A-F-T-E-C-C |
| B7 | T-E-F-A-K-T-C-V-A-D-E-S-A-E-N | D2 | F-A-K-R-Y-K-A-A-F-T-E-C-C-Q-A |
| B8 | F-A-K-T-C-V-A-D-E-S-A-E-N-C-D | D3 | K-R-Y-K-A-A-F-T-E-C-C-Q-A-A-D |
| B9 | K-T-C-V-A-D-E-S-A-E-N-C-D-K-S | D4 | Y-K-A-A-F-T-E-C-C-Q-A-A-D-K-A |
| B10 | C-V-A-D-E-S-A-E-N-C-D-K-S-L-H | D5 | A-A-F-T-E-C-C-Q-A-A-D-K-A-A-C |
| B11 | A-D-E-S-A-E-N-C-D-K-S-L-H-T-L | D6 | F-T-E-C-C-Q-A-A-D-K-A-A-C-L-L |
| B12 | E-S-A-E-N-C-D-K-S-L-H-T-L-F-G | D7 | E-C-C-Q-A-A-D-K-A-A-C-L-L-P-K |
| B13 | A-E-N-C-D-K-S-L-H-T-L-F-G-D-K | D8 | C-Q-A-A-D-K-A-A-C-L-L-P-K-L-D |
| B14 | N-C-D-K-S-L-H-T-L-F-G-D-K-L-C | D9 | A-A-D-K-A-A-C-L-L-P-K-L-D-E-L |
| B15 | D-K-S-L-H-T-L-F-G-D-K-L-C-T-V | D10 | D-K-A-A-C-L-L-P-K-L-D-E-L-R-D |
| B16 | S-L-H-T-L-F-G-D-K-L-C-T-V-A-T | D11 | A-A-C-L-L-P-K-L-D-E-L-R-D-E-G |
| B17 | H-T-L-F-G-D-K-L-C-T-V-A-T-L-R | D12 | C-L-L-P-K-L-D-E-L-R-D-E-G-K-A |
| B18 | L-F-G-D-K-L-C-T-V-A-T-L-R-E-T | D13 | L-P-K-L-D-E-L-R-D-E-G-K-A-S-S |
| B19 | G-D-K-L-C-T-V-A-T-L-R-E-T-Y-G | D14 | K-L-D-E-L-R-D-E-G-K-A-S-S-A-K |
| B20 | K-L-C-T-V-A-T-L-R-E-T-Y-G-E-M | D15 | D-E-L-R-D-E-G-K-A-S-S-A-K-Q-R |
| B21 | C-T-V-A-T-L-R-E-T-Y-G-E-M-A-D | D16 | L-R-D-E-G-K-A-S-S-A-K-Q-R-L-K |
| B22 | V-A-T-L-R-E-T-Y-G-E-M-A-D-C-C | D17 | D-E-G-K-A-S-S-A-K-Q-R-L-K-C-A |
| B23 | T-L-R-E-T-Y-G-E-M-A-D-C-C-A-K | D18 | G-K-A-S-S-A-K-Q-R-L-K-C-A-S-L |
| B24 | R-E-T-Y-G-E-M-A-D-C-C-A-K-Q-E | D19 | A-S-S-A-K-Q-R-L-K-C-A-S-L-Q-K |
| B25 | T-Y-G-E-M-A-D-C-C-A-K-Q-E-P-E | D20 | S-A-K-Q-R-L-K-C-A-S-L-Q-K-F-G |

|  |  |  |  |
| --- | --- | --- | --- |
| **Positional Reference** | **Peptide sequence** | **Positional Reference** | **Peptide sequence** |
| D21 | K-Q-R-L-K-C-A-S-L-Q-K-F-G-E-R | F16 | L-A-A-D-F-V-E-S-K-D-V-C-K-N-Y |
| D22 | R-L-K-C-A-S-L-Q-K-F-G-E-R-A-F | F17 | A-D-F-V-E-S-K-D-V-C-K-N-Y-A-E |
| D23 | K-C-A-S-L-Q-K-F-G-E-R-A-F-K-A | F18 | F-V-E-S-K-D-V-C-K-N-Y-A-E-A-K |
| D24 | A-S-L-Q-K-F-G-E-R-A-F-K-A-W-A | F19 | E-S-K-D-V-C-K-N-Y-A-E-A-K-D-V |
| D25 | L-Q-K-F-G-E-R-A-F-K-A-W-A-V-A | F20 | K-D-V-C-K-N-Y-A-E-A-K-D-V-F-L |
| D26 | K-F-G-E-R-A-F-K-A-W-A-V-A-R-L | F21 | V-C-K-N-Y-A-E-A-K-D-V-F-L-G-M |
| D27 | G-E-R-A-F-K-A-W-A-V-A-R-L-S-Q | F22 | K-N-Y-A-E-A-K-D-V-F-L-G-M-F-L |
| D28 | R-A-F-K-A-W-A-V-A-R-L-S-Q-R-F | F23 | Y-A-E-A-K-D-V-F-L-G-M-F-L-Y-E |
| D29 | F-K-A-W-A-V-A-R-L-S-Q-R-F-P-K | F24 | E-A-K-D-V-F-L-G-M-F-L-Y-E-Y-A |
| D30 | A-W-A-V-A-R-L-S-Q-R-F-P-K-A-E | F25 | K-D-V-F-L-G-M-F-L-Y-E-Y-A-R-R |
| E1 | A-V-A-R-L-S-Q-R-F-P-K-A-E-F-A | F26 | V-F-L-G-M-F-L-Y-E-Y-A-R-R-H-P |
| E2 | A-R-L-S-Q-R-F-P-K-A-E-F-A-E-V | F27 | L-G-M-F-L-Y-E-Y-A-R-R-H-P-D-Y |
| E3 | L-S-Q-R-F-P-K-A-E-F-A-E-V-S-K | F28 | M-F-L-Y-E-Y-A-R-R-H-P-D-Y-S-V |
| E4 | Q-R-F-P-K-A-E-F-A-E-V-S-K-L-V | F29 | L-Y-E-Y-A-R-R-H-P-D-Y-S-V-V-L |
| E5 | F-P-K-A-E-F-A-E-V-S-K-L-V-T-D | F30 | E-Y-A-R-R-H-P-D-Y-S-V-V-L-L-L |
| E6 | K-A-E-F-A-E-V-S-K-L-V-T-D-L-T | G1 | A-R-R-H-P-D-Y-S-V-V-L-L-L-R-L |
| E7 | E-F-A-E-V-S-K-L-V-T-D-L-T-K-V | G2 | R-H-P-D-Y-S-V-V-L-L-L-R-L-A-K |
| E8 | A-E-V-S-K-L-V-T-D-L-T-K-V-H-T | G3 | P-D-Y-S-V-V-L-L-L-R-L-A-K-T-Y |
| E9 | V-S-K-L-V-T-D-L-T-K-V-H-T-E-C | G4 | Y-S-V-V-L-L-L-R-L-A-K-T-Y-E-T |
| E10 | K-L-V-T-D-L-T-K-V-H-T-E-C-C-H | G5 | V-V-L-L-L-R-L-A-K-T-Y-E-T-T-L |
| E11 | V-T-D-L-T-K-V-H-T-E-C-C-H-G-D | G6 | L-L-L-R-L-A-K-T-Y-E-T-T-L-E-K |
| E12 | D-L-T-K-V-H-T-E-C-C-H-G-D-L-L | G7 | L-R-L-A-K-T-Y-E-T-T-L-E-K-C-C |
| E13 | T-K-V-H-T-E-C-C-H-G-D-L-L-E-C | G8 | L-A-K-T-Y-E-T-T-L-E-K-C-C-A-A |
| E14 | V-H-T-E-C-C-H-G-D-L-L-E-C-A-D | G9 | K-T-Y-E-T-T-L-E-K-C-C-A-A-A-D |
| E15 | T-E-C-C-H-G-D-L-L-E-C-A-D-D-R | G10 | Y-E-T-T-L-E-K-C-C-A-A-A-D-P-H |
| E16 | C-C-H-G-D-L-L-E-C-A-D-D-R-A-D | G11 | T-T-L-E-K-C-C-A-A-A-D-P-H-E-C |
| E17 | H-G-D-L-L-E-C-A-D-D-R-A-D-L-A | G12 | L-E-K-C-C-A-A-A-D-P-H-E-C-Y-A |
| E18 | D-L-L-E-C-A-D-D-R-A-D-L-A-K-Y | G13 | K-C-C-A-A-A-D-P-H-E-C-Y-A-K-V |
| E19 | L-E-C-A-D-D-R-A-D-L-A-K-Y-I-C | G14 | C-A-A-A-D-P-H-E-C-Y-A-K-V-F-D |
| E20 | C-A-D-D-R-A-D-L-A-K-Y-I-C-E-N | G15 | A-A-D-P-H-E-C-Y-A-K-V-F-D-E-F |
| E21 | D-D-R-A-D-L-A-K-Y-I-C-E-N-Q-D | G16 | D-P-H-E-C-Y-A-K-V-F-D-E-F-K-P |
| E22 | R-A-D-L-A-K-Y-I-C-E-N-Q-D-S-I | G17 | H-E-C-Y-A-K-V-F-D-E-F-K-P-L-V |
| E23 | D-L-A-K-Y-I-C-E-N-Q-D-S-I-S-S | G18 | C-Y-A-K-V-F-D-E-F-K-P-L-V-E-E |
| E24 | A-K-Y-I-C-E-N-Q-D-S-I-S-S-K-L | G19 | A-K-V-F-D-E-F-K-P-L-V-E-E-P-Q |
| E25 | Y-I-C-E-N-Q-D-S-I-S-S-K-L-K-E | G20 | V-F-D-E-F-K-P-L-V-E-E-P-Q-N-L |
| E26 | C-E-N-Q-D-S-I-S-S-K-L-K-E-C-C | G21 | D-E-F-K-P-L-V-E-E-P-Q-N-L-I-K |
| E27 | N-Q-D-S-I-S-S-K-L-K-E-C-C-E-K | G22 | F-K-P-L-V-E-E-P-Q-N-L-I-K-Q-N |
| E28 | D-S-I-S-S-K-L-K-E-C-C-E-K-P-L | G23 | P-L-V-E-E-P-Q-N-L-I-K-Q-N-C-E |
| E29 | I-S-S-K-L-K-E-C-C-E-K-P-L-L-E | G24 | V-E-E-P-Q-N-L-I-K-Q-N-C-E-L-F |
| E30 | S-K-L-K-E-C-C-E-K-P-L-L-E-K-S | G25 | E-P-Q-N-L-I-K-Q-N-C-E-L-F-E-Q |
| F1 | L-K-E-C-C-E-K-P-L-L-E-K-S-H-C | G26 | Q-N-L-I-K-Q-N-C-E-L-F-E-Q-L-G |
| F2 | E-C-C-E-K-P-L-L-E-K-S-H-C-I-A | G27 | L-I-K-Q-N-C-E-L-F-E-Q-L-G-E-Y |
| F3 | C-E-K-P-L-L-E-K-S-H-C-I-A-E-V | G28 | K-Q-N-C-E-L-F-E-Q-L-G-E-Y-K-F |
| F4 | K-P-L-L-E-K-S-H-C-I-A-E-V-E-N | G29 | N-C-E-L-F-E-Q-L-G-E-Y-K-F-Q-N |
| F5 | L-L-E-K-S-H-C-I-A-E-V-E-N-D-E | G30 | E-L-F-E-Q-L-G-E-Y-K-F-Q-N-A-L |
| F6 | E-K-S-H-C-I-A-E-V-E-N-D-E-M-P | H1 | F-E-Q-L-G-E-Y-K-F-Q-N-A-L-L-V |
| F7 | S-H-C-I-A-E-V-E-N-D-E-M-P-A-D | H2 | Q-L-G-E-Y-K-F-Q-N-A-L-L-V-R-Y |
| F8 | C-I-A-E-V-E-N-D-E-M-P-A-D-L-P | H3 | G-E-Y-K-F-Q-N-A-L-L-V-R-Y-T-K |
| F9 | A-E-V-E-N-D-E-M-P-A-D-L-P-S-L | H4 | Y-K-F-Q-N-A-L-L-V-R-Y-T-K-K-V |
| F10 | V-E-N-D-E-M-P-A-D-L-P-S-L-A-A | H5 | F-Q-N-A-L-L-V-R-Y-T-K-K-V-P-Q |
| F11 | N-D-E-M-P-A-D-L-P-S-L-A-A-D-F | H6 | N-A-L-L-V-R-Y-T-K-K-V-P-Q-V-S |
| F12 | E-M-P-A-D-L-P-S-L-A-A-D-F-V-E | H7 | L-L-V-R-Y-T-K-K-V-P-Q-V-S-T-P |
| F13 | P-A-D-L-P-S-L-A-A-D-F-V-E-S-K | H8 | V-R-Y-T-K-K-V-P-Q-V-S-T-P-T-L |
| F14 | D-L-P-S-L-A-A-D-F-V-E-S-K-D-V | H9 | Y-T-K-K-V-P-Q-V-S-T-P-T-L-V-E |
| F15 | P-S-L-A-A-D-F-V-E-S-K-D-V-C-K | H10 | K-K-V-P-Q-V-S-T-P-T-L-V-E-V-S |

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| **Positional Reference** | **Peptide sequence** | **Positional Reference** | **Peptide sequence** |
| H11 | V-P-Q-V-S-T-P-T-L-V-E-V-S-R-N | J6 | K-Q-T-A-L-V-E-L-V-K-H-K-P-K-A |
| H12 | Q-V-S-T-P-T-L-V-E-V-S-R-N-L-G | J7 | T-A-L-V-E-L-V-K-H-K-P-K-A-T-K |
| H13 | S-T-P-T-L-V-E-V-S-R-N-L-G-K-V | J8 | L-V-E-L-V-K-H-K-P-K-A-T-K-E-Q |
| H14 | P-T-L-V-E-V-S-R-N-L-G-K-V-G-S | J9 | E-L-V-K-H-K-P-K-A-T-K-E-Q-L-K |
| H15 | L-V-E-V-S-R-N-L-G-K-V-G-S-K-C | J10 | V-K-H-K-P-K-A-T-K-E-Q-L-K-A-V |
| H16 | E-V-S-R-N-L-G-K-V-G-S-K-C-C-K | J11 | H-K-P-K-A-T-K-E-Q-L-K-A-V-M-D |
| H17 | S-R-N-L-G-K-V-G-S-K-C-C-K-H-P | J12 | P-K-A-T-K-E-Q-L-K-A-V-M-D-D-F |
| H18 | N-L-G-K-V-G-S-K-C-C-K-H-P-E-A | J13 | A-T-K-E-Q-L-K-A-V-M-D-D-F-A-A |
| H19 | G-K-V-G-S-K-C-C-K-H-P-E-A-K-R | J14 | K-E-Q-L-K-A-V-M-D-D-F-A-A-F-V |
| H20 | V-G-S-K-C-C-K-H-P-E-A-K-R-M-P | J15 | Q-L-K-A-V-M-D-D-F-A-A-F-V-E-K |
| H21 | S-K-C-C-K-H-P-E-A-K-R-M-P-C-A | J16 | K-A-V-M-D-D-F-A-A-F-V-E-K-C-C |
| H22 | C-C-K-H-P-E-A-K-R-M-P-C-A-E-D | J17 | V-M-D-D-F-A-A-F-V-E-K-C-C-K-A |
| H23 | K-H-P-E-A-K-R-M-P-C-A-E-D-Y-L | J18 | D-D-F-A-A-F-V-E-K-C-C-K-A-D-D |
| H24 | P-E-A-K-R-M-P-C-A-E-D-Y-L-S-V | J19 | F-A-A-F-V-E-K-C-C-K-A-D-D-K-E |
| H25 | A-K-R-M-P-C-A-E-D-Y-L-S-V-V-L | J20 | A-F-V-E-K-C-C-K-A-D-D-K-E-T-C |
| H26 | R-M-P-C-A-E-D-Y-L-S-V-V-L-N-Q | J21 | V-E-K-C-C-K-A-D-D-K-E-T-C-F-A |
| H27 | P-C-A-E-D-Y-L-S-V-V-L-N-Q-L-C | J22 | K-C-C-K-A-D-D-K-E-T-C-F-A-E-E |
| H28 | A-E-D-Y-L-S-V-V-L-N-Q-L-C-V-L | J23 | C-K-A-D-D-K-E-T-C-F-A-E-E-G-K |
| H29 | D-Y-L-S-V-V-L-N-Q-L-C-V-L-H-E | J24 | A-D-D-K-E-T-C-F-A-E-E-G-K-K-L |
| H30 | L-S-V-V-L-N-Q-L-C-V-L-H-E-K-T | J25 | D-K-E-T-C-F-A-E-E-G-K-K-L-V-A |
| I1 | V-V-L-N-Q-L-C-V-L-H-E-K-T-P-V | J26 | E-T-C-F-A-E-E-G-K-K-L-V-A-A-S |
| I2 | L-N-Q-L-C-V-L-H-E-K-T-P-V-S-D | J27 | C-F-A-E-E-G-K-K-L-V-A-A-S-Q-A |
| I3 | Q-L-C-V-L-H-E-K-T-P-V-S-D-R-V | J28 | A-E-E-G-K-K-L-V-A-A-S-Q-A-A-L |
| I4 | C-V-L-H-E-K-T-P-V-S-D-R-V-T-K | J29 | E-G-K-K-L-V-A-A-S-Q-A-A-L-G-L |
| I5 | L-H-E-K-T-P-V-S-D-R-V-T-K-C-C | J30 | Blank |
| I6 | E-K-T-P-V-S-D-R-V-T-K-C-C-T-E |
| I7 | T-P-V-S-D-R-V-T-K-C-C-T-E-S-L |
| I8 | V-S-D-R-V-T-K-C-C-T-E-S-L-V-N |
| I9 | D-R-V-T-K-C-C-T-E-S-L-V-N-R-R |
| I10 | V-T-K-C-C-T-E-S-L-V-N-R-R-P-C |
| I11 | K-C-C-T-E-S-L-V-N-R-R-P-C-F-S |
| I12 | C-T-E-S-L-V-N-R-R-P-C-F-S-A-L |
| I13 | E-S-L-V-N-R-R-P-C-F-S-A-L-E-V |
| I14 | L-V-N-R-R-P-C-F-S-A-L-E-V-D-E |
| I15 | N-R-R-P-C-F-S-A-L-E-V-D-E-T-Y |
| I16 | R-P-C-F-S-A-L-E-V-D-E-T-Y-V-P |
| I17 | C-F-S-A-L-E-V-D-E-T-Y-V-P-K-E |
| I18 | S-A-L-E-V-D-E-T-Y-V-P-K-E-F-N |
| I19 | L-E-V-D-E-T-Y-V-P-K-E-F-N-A-E |
| I20 | V-D-E-T-Y-V-P-K-E-F-N-A-E-T-F |
| I21 | E-T-Y-V-P-K-E-F-N-A-E-T-F-T-F |
| I22 | Y-V-P-K-E-F-N-A-E-T-F-T-F-H-A |
| I23 | P-K-E-F-N-A-E-T-F-T-F-H-A-D-I |
| I24 | E-F-N-A-E-T-F-T-F-H-A-D-I-C-T |
| I25 | N-A-E-T-F-T-F-H-A-D-I-C-T-L-S |
| I26 | E-T-F-T-F-H-A-D-I-C-T-L-S-E-K |
| I27 | F-T-F-H-A-D-I-C-T-L-S-E-K-E-R |
| I28 | F-H-A-D-I-C-T-L-S-E-K-E-R-Q-I |
| I29 | A-D-I-C-T-L-S-E-K-E-R-Q-I-K-K |
| I30 | I-C-T-L-S-E-K-E-R-Q-I-K-K-Q-T |
| J1 | T-L-S-E-K-E-R-Q-I-K-K-Q-T-A-L |
| J2 | S-E-K-E-R-Q-I-K-K-Q-T-A-L-V-E |
| J3 | K-E-R-Q-I-K-K-Q-T-A-L-V-E-L-V |
| J4 | R-Q-I-K-K-Q-T-A-L-V-E-L-V-K-H |
| J5 | I-K-K-Q-T-A-L-V-E-L-V-K-H-K-P |

**Supplementary Table 2.**  List of the ovalbumin (UniProt ID: P01012) 15-mer peptides synthesized on the arrays.

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| **Positional**  **Reference** | **Peptide sequence** | **Positional**  **Reference** | **Peptide sequence** |
| A1 | Blank | B26 | E-E-R-Y-P-I-L-P-E-Y-L-Q-C-V-K |
| A2 | M-G-S-I-G-A-A-S-M-E-F-C-F-D-V | B27 | R-Y-P-I-L-P-E-Y-L-Q-C-V-K-E-L |
| A3 | S-I-G-A-A-S-M-E-F-C-F-D-V-F-K | B28 | P-I-L-P-E-Y-L-Q-C-V-K-E-L-Y-R |
| A4 | G-A-A-S-M-E-F-C-F-D-V-F-K-E-L | B29 | L-P-E-Y-L-Q-C-V-K-E-L-Y-R-G-G |
| A5 | A-S-M-E-F-C-F-D-V-F-K-E-L-K-V | B30 | E-Y-L-Q-C-V-K-E-L-Y-R-G-G-L-E |
| A6 | M-E-F-C-F-D-V-F-K-E-L-K-V-H-H | C1 | L-Q-C-V-K-E-L-Y-R-G-G-L-E-P-I |
| A7 | F-C-F-D-V-F-K-E-L-K-V-H-H-A-N | C2 | C-V-K-E-L-Y-R-G-G-L-E-P-I-N-F |
| A8 | F-D-V-F-K-E-L-K-V-H-H-A-N-E-N | C3 | K-E-L-Y-R-G-G-L-E-P-I-N-F-Q-T |
| A9 | V-F-K-E-L-K-V-H-H-A-N-E-N-I-F | C4 | L-Y-R-G-G-L-E-P-I-N-F-Q-T-A-A |
| A10 | K-E-L-K-V-H-H-A-N-E-N-I-F-Y-C | C5 | R-G-G-L-E-P-I-N-F-Q-T-A-A-D-Q |
| A11 | L-K-V-H-H-A-N-E-N-I-F-Y-C-P-I | C6 | G-L-E-P-I-N-F-Q-T-A-A-D-Q-A-R |
| A12 | V-H-H-A-N-E-N-I-F-Y-C-P-I-A-I | C7 | E-P-I-N-F-Q-T-A-A-D-Q-A-R-E-L |
| A13 | H-A-N-E-N-I-F-Y-C-P-I-A-I-M-S | C8 | I-N-F-Q-T-A-A-D-Q-A-R-E-L-I-N |
| A14 | N-E-N-I-F-Y-C-P-I-A-I-M-S-A-L | C9 | F-Q-T-A-A-D-Q-A-R-E-L-I-N-S-W |
| A15 | N-I-F-Y-C-P-I-A-I-M-S-A-L-A-M | C10 | T-A-A-D-Q-A-R-E-L-I-N-S-W-V-E |
| A16 | F-Y-C-P-I-A-I-M-S-A-L-A-M-V-Y | C11 | A-D-Q-A-R-E-L-I-N-S-W-V-E-S-Q |
| A17 | C-P-I-A-I-M-S-A-L-A-M-V-Y-L-G | C12 | Q-A-R-E-L-I-N-S-W-V-E-S-Q-T-N |
| A18 | I-A-I-M-S-A-L-A-M-V-Y-L-G-A-K | C13 | R-E-L-I-N-S-W-V-E-S-Q-T-N-G-I |
| A19 | I-M-S-A-L-A-M-V-Y-L-G-A-K-D-S | C14 | L-I-N-S-W-V-E-S-Q-T-N-G-I-I-R |
| A20 | S-A-L-A-M-V-Y-L-G-A-K-D-S-T-R | C15 | N-S-W-V-E-S-Q-T-N-G-I-I-R-N-V |
| A21 | L-A-M-V-Y-L-G-A-K-D-S-T-R-T-Q | C16 | W-V-E-S-Q-T-N-G-I-I-R-N-V-L-Q |
| A22 | M-V-Y-L-G-A-K-D-S-T-R-T-Q-I-N | C17 | E-S-Q-T-N-G-I-I-R-N-V-L-Q-P-S |
| A23 | Y-L-G-A-K-D-S-T-R-T-Q-I-N-K-V | C18 | Q-T-N-G-I-I-R-N-V-L-Q-P-S-S-V |
| A24 | G-A-K-D-S-T-R-T-Q-I-N-K-V-V-R | C19 | N-G-I-I-R-N-V-L-Q-P-S-S-V-D-S |
| A25 | K-D-S-T-R-T-Q-I-N-K-V-V-R-F-D | C20 | I-I-R-N-V-L-Q-P-S-S-V-D-S-Q-T |
| A26 | S-T-R-T-Q-I-N-K-V-V-R-F-D-K-L | C21 | R-N-V-L-Q-P-S-S-V-D-S-Q-T-A-M |
| A27 | R-T-Q-I-N-K-V-V-R-F-D-K-L-P-G | C22 | V-L-Q-P-S-S-V-D-S-Q-T-A-M-V-L |
| A28 | Q-I-N-K-V-V-R-F-D-K-L-P-G-F-G | C23 | Q-P-S-S-V-D-S-Q-T-A-M-V-L-V-N |
| A29 | N-K-V-V-R-F-D-K-L-P-G-F-G-D-S | C24 | S-S-V-D-S-Q-T-A-M-V-L-V-N-A-I |
| A30 | V-V-R-F-D-K-L-P-G-F-G-D-S-I-E | C25 | V-D-S-Q-T-A-M-V-L-V-N-A-I-V-F |
| B1 | R-F-D-K-L-P-G-F-G-D-S-I-E-A-Q | C26 | S-Q-T-A-M-V-L-V-N-A-I-V-F-K-G |
| B2 | D-K-L-P-G-F-G-D-S-I-E-A-Q-C-G | C27 | T-A-M-V-L-V-N-A-I-V-F-K-G-L-W |
| B3 | L-P-G-F-G-D-S-I-E-A-Q-C-G-T-S | C28 | M-V-L-V-N-A-I-V-F-K-G-L-W-E-K |
| B4 | G-F-G-D-S-I-E-A-Q-C-G-T-S-V-N | C29 | L-V-N-A-I-V-F-K-G-L-W-E-K-A-F |
| B5 | G-D-S-I-E-A-Q-C-G-T-S-V-N-V-H | C30 | N-A-I-V-F-K-G-L-W-E-K-A-F-K-D |
| B6 | S-I-E-A-Q-C-G-T-S-V-N-V-H-S-S | D1 | I-V-F-K-G-L-W-E-K-A-F-K-D-E-D |
| B7 | E-A-Q-C-G-T-S-V-N-V-H-S-S-L-R | D2 | F-K-G-L-W-E-K-A-F-K-D-E-D-T-Q |
| B8 | Q-C-G-T-S-V-N-V-H-S-S-L-R-D-I | D3 | G-L-W-E-K-A-F-K-D-E-D-T-Q-A-M |
| B9 | G-T-S-V-N-V-H-S-S-L-R-D-I-L-N | D4 | W-E-K-A-F-K-D-E-D-T-Q-A-M-P-F |
| B10 | S-V-N-V-H-S-S-L-R-D-I-L-N-Q-I | D5 | K-A-F-K-D-E-D-T-Q-A-M-P-F-R-V |
| B11 | N-V-H-S-S-L-R-D-I-L-N-Q-I-T-K | D6 | F-K-D-E-D-T-Q-A-M-P-F-R-V-T-E |
| B12 | H-S-S-L-R-D-I-L-N-Q-I-T-K-P-N | D7 | D-E-D-T-Q-A-M-P-F-R-V-T-E-Q-E |
| B13 | S-L-R-D-I-L-N-Q-I-T-K-P-N-D-V | D8 | D-T-Q-A-M-P-F-R-V-T-E-Q-E-S-K |
| B14 | R-D-I-L-N-Q-I-T-K-P-N-D-V-Y-S | D9 | Q-A-M-P-F-R-V-T-E-Q-E-S-K-P-V |
| B15 | I-L-N-Q-I-T-K-P-N-D-V-Y-S-F-S | D10 | M-P-F-R-V-T-E-Q-E-S-K-P-V-Q-M |
| B16 | N-Q-I-T-K-P-N-D-V-Y-S-F-S-L-A | D11 | F-R-V-T-E-Q-E-S-K-P-V-Q-M-M-Y |
| B17 | I-T-K-P-N-D-V-Y-S-F-S-L-A-S-R | D12 | V-T-E-Q-E-S-K-P-V-Q-M-M-Y-Q-I |
| B18 | K-P-N-D-V-Y-S-F-S-L-A-S-R-L-Y | D13 | E-Q-E-S-K-P-V-Q-M-M-Y-Q-I-G-L |
| B19 | N-D-V-Y-S-F-S-L-A-S-R-L-Y-A-E | D14 | E-S-K-P-V-Q-M-M-Y-Q-I-G-L-F-R |
| B20 | V-Y-S-F-S-L-A-S-R-L-Y-A-E-E-R | D15 | K-P-V-Q-M-M-Y-Q-I-G-L-F-R-V-A |
| B21 | S-F-S-L-A-S-R-L-Y-A-E-E-R-Y-P | D16 | V-Q-M-M-Y-Q-I-G-L-F-R-V-A-S-M |
| B22 | S-L-A-S-R-L-Y-A-E-E-R-Y-P-I-L | D17 | M-M-Y-Q-I-G-L-F-R-V-A-S-M-A-S |
| B23 | A-S-R-L-Y-A-E-E-R-Y-P-I-L-P-E | D18 | Y-Q-I-G-L-F-R-V-A-S-M-A-S-E-K |
| B24 | R-L-Y-A-E-E-R-Y-P-I-L-P-E-Y-L | D19 | I-G-L-F-R-V-A-S-M-A-S-E-K-M-K |
| B25 | Y-A-E-E-R-Y-P-I-L-P-E-Y-L-Q-C | D20 | L-F-R-V-A-S-M-A-S-E-K-M-K-I-L |

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| **Positional Reference** | **Peptide sequence** | **Positional Reference** | **Peptide sequence** |
| D21 | R-V-A-S-M-A-S-E-K-M-K-I-L-E-L | F16 | H-A-A-H-A-E-I-N-E-A-G-R-E-V-V |
| D22 | A-S-M-A-S-E-K-M-K-I-L-E-L-P-F | F17 | A-H-A-E-I-N-E-A-G-R-E-V-V-G-S |
| D23 | M-A-S-E-K-M-K-I-L-E-L-P-F-A-S | F18 | A-E-I-N-E-A-G-R-E-V-V-G-S-A-E |
| D24 | S-E-K-M-K-I-L-E-L-P-F-A-S-G-T | F19 | I-N-E-A-G-R-E-V-V-G-S-A-E-A-G |
| D25 | K-M-K-I-L-E-L-P-F-A-S-G-T-M-S | F20 | E-A-G-R-E-V-V-G-S-A-E-A-G-V-D |
| D26 | K-I-L-E-L-P-F-A-S-G-T-M-S-M-L | F21 | G-R-E-V-V-G-S-A-E-A-G-V-D-A-A |
| D27 | L-E-L-P-F-A-S-G-T-M-S-M-L-V-L | F22 | E-V-V-G-S-A-E-A-G-V-D-A-A-S-V |
| D28 | L-P-F-A-S-G-T-M-S-M-L-V-L-L-P | F23 | V-G-S-A-E-A-G-V-D-A-A-S-V-S-E |
| D29 | F-A-S-G-T-M-S-M-L-V-L-L-P-D-E | F24 | S-A-E-A-G-V-D-A-A-S-V-S-E-E-F |
| D30 | S-G-T-M-S-M-L-V-L-L-P-D-E-V-S | F25 | E-A-G-V-D-A-A-S-V-S-E-E-F-R-A |
| E1 | T-M-S-M-L-V-L-L-P-D-E-V-S-G-L | F26 | G-V-D-A-A-S-V-S-E-E-F-R-A-D-H |
| E2 | S-M-L-V-L-L-P-D-E-V-S-G-L-E-Q | F27 | D-A-A-S-V-S-E-E-F-R-A-D-H-P-F |
| E3 | L-V-L-L-P-D-E-V-S-G-L-E-Q-L-E | F28 | A-S-V-S-E-E-F-R-A-D-H-P-F-L-F |
| E4 | L-L-P-D-E-V-S-G-L-E-Q-L-E-S-I | F29 | V-S-E-E-F-R-A-D-H-P-F-L-F-C-I |
| E5 | P-D-E-V-S-G-L-E-Q-L-E-S-I-I-N | F30 | E-E-F-R-A-D-H-P-F-L-F-C-I-K-H |
| E6 | E-V-S-G-L-E-Q-L-E-S-I-I-N-F-E | G1 | F-R-A-D-H-P-F-L-F-C-I-K-H-I-A |
| E7 | S-G-L-E-Q-L-E-S-I-I-N-F-E-K-L | G2 | A-D-H-P-F-L-F-C-I-K-H-I-A-T-N |
| E8 | L-E-Q-L-E-S-I-I-N-F-E-K-L-T-E | G3 | H-P-F-L-F-C-I-K-H-I-A-T-N-A-V |
| E9 | Q-L-E-S-I-I-N-F-E-K-L-T-E-W-T | G4 | F-L-F-C-I-K-H-I-A-T-N-A-V-L-F |
| E10 | E-S-I-I-N-F-E-K-L-T-E-W-T-S-S | G5 | F-C-I-K-H-I-A-T-N-A-V-L-F-F-G |
| E11 | I-I-N-F-E-K-L-T-E-W-T-S-S-N-V | G6 | I-K-H-I-A-T-N-A-V-L-F-F-G-R-C |
| E12 | N-F-E-K-L-T-E-W-T-S-S-N-V-M-E | G7 | H-I-A-T-N-A-V-L-F-F-G-R-C-V-S |
| E13 | E-K-L-T-E-W-T-S-S-N-V-M-E-E-R | G8 | A-T-N-A-V-L-F-F-G-R-C-V-S-P |
| E14 | L-T-E-W-T-S-S-N-V-M-E-E-R-K-I |  |  |
| E15 | E-W-T-S-S-N-V-M-E-E-R-K-I-K-V |  |  |
| E16 | T-S-S-N-V-M-E-E-R-K-I-K-V-Y-L |  |  |
| E17 | S-N-V-M-E-E-R-K-I-K-V-Y-L-P-R |  |  |
| E18 | V-M-E-E-R-K-I-K-V-Y-L-P-R-M-K |  |  |
| E19 | E-E-R-K-I-K-V-Y-L-P-R-M-K-M-E |  |  |
| E20 | R-K-I-K-V-Y-L-P-R-M-K-M-E-E-K |  |  |
| E21 | I-K-V-Y-L-P-R-M-K-M-E-E-K-Y-N |  |  |
| E22 | V-Y-L-P-R-M-K-M-E-E-K-Y-N-L-T |  |  |
| E23 | L-P-R-M-K-M-E-E-K-Y-N-L-T-S-V |  |  |
| E24 | R-M-K-M-E-E-K-Y-N-L-T-S-V-L-M |  |  |
| E25 | K-M-E-E-K-Y-N-L-T-S-V-L-M-A-M |  |  |
| E26 | E-E-K-Y-N-L-T-S-V-L-M-A-M-G-I |  |  |
| E27 | K-Y-N-L-T-S-V-L-M-A-M-G-I-T-D |  |  |
| E28 | N-L-T-S-V-L-M-A-M-G-I-T-D-V-F |  |  |
| E29 | T-S-V-L-M-A-M-G-I-T-D-V-F-S-S |  |  |
| E30 | V-L-M-A-M-G-I-T-D-V-F-S-S-S-A |  |  |
| F1 | M-A-M-G-I-T-D-V-F-S-S-S-A-N-L |  |  |
| F2 | M-G-I-T-D-V-F-S-S-S-A-N-L-S-G |  |  |
| F3 | I-T-D-V-F-S-S-S-A-N-L-S-G-I-S |  |  |
| F4 | D-V-F-S-S-S-A-N-L-S-G-I-S-S-A |  |  |
| F5 | F-S-S-S-A-N-L-S-G-I-S-S-A-E-S |  |  |
| F6 | S-S-A-N-L-S-G-I-S-S-A-E-S-L-K |  |  |
| F7 | A-N-L-S-G-I-S-S-A-E-S-L-K-I-S |  |  |
| F8 | L-S-G-I-S-S-A-E-S-L-K-I-S-Q-A |  |  |
| F9 | G-I-S-S-A-E-S-L-K-I-S-Q-A-V-H |  |  |
| F10 | S-S-A-E-S-L-K-I-S-Q-A-V-H-A-A |  |  |
| F11 | A-E-S-L-K-I-S-Q-A-V-H-A-A-H-A |  |  |
| F12 | S-L-K-I-S-Q-A-V-H-A-A-H-A-E-I |  |  |
| F13 | K-I-S-Q-A-V-H-A-A-H-A-E-I-N-E |  |  |
| F14 | S-Q-A-V-H-A-A-H-A-E-I-N-E-A-G |  |  |
| F15 | A-V-H-A-A-H-A-E-I-N-E-A-G-R-E |  |  |