

## Lindberg Lab Antisera Descriptions (2/16, IL)

The first number (bolded) is the rabbit number; on each tube, it will be followed by a B for Bleed, then the second number, which is variable since it is the bleed number. F denotes the Final bleed. All numbered antisera (except numbers 45 and 46 and s43 and s44) are raised to peptides 10-15 residues in length, usually c-terminal, conjugated to keyhole limpet hemocyanin (Pierce Chem) with ECDI (with some exceptions). Starred rabbits are preferred, though if you need a lot of antiserum ask us for the other one!

Please look up the full reference (in the CV listed on this same website) and CITE IT when using one of these antisera. Thanks!

Note after Katrina- samples of all antisera were rescued at three weeks in our famous guerrilla raid. While many were moldy, some had azide and were fine (you can guess what the moral is here). We have partially purified IgGs from about half of these antisera using ammonium sulfate precipitation. We did this to decrease the number of small bottles from different bleeds, which were taking up too much space. Both previously moldy and non-moldy antisera appear to work well for blotting; other uses have not been tested thus far, but they will probably be fine. We also have certain, but not most, preimmune sera which suffered identical fates.

**1\* & 2:** Mouse PC1, mature N-terminus. 2 is most commonly used (also almost out)- Good for immunoppt and Western blotting; ICC; RIA titer over a million. Reference Vindrola and Lindberg Mol. Endo. 1992 and Hornby et al Neuroendocrinol. 1993 (for ICC). Affinity Bioreagents carries a similar antiserum, tested by our lab. The peptide immunogen has been supplied to the Mains and Devi labs and they have raised similar antisera. Affinity Bioreagents carries a similar antiserum raised using our antigen and tested by our lab.

**3\*:** Mouse PC1 C-terminus- this one does not see the 66 kDa form. Vindrola and Lindberg Mol. Endo. 1992 and Hornby et al Neuroendocrinol. 1993 (for ICC).

**4\* & 5:** Mouse PC2 C-terminus. ([YERSLQSLRKN coupled to succinylated hemocyanin](#)). Supplanted by 18 because we ran out. Good for immunoppt, Western blotting and ICC. Reference Shen et al, 1993. Note that Affinity Bioreagents carries a similar antiserum tested by our lab.

**6 & 7\*:** Mouse PC2 mature N-terminus. Reference Shen et al, 1993.

**12 & 13\*:** same 7B2 epitope originally used by the Seidah group (internal, 21 kDa). All vertebrate species. Blots recombinant stuff, but not as well as the Martens monoclonal. Good for RIA, Immunoppt. Reference Zhu and Lindberg 1994. Note- it is VERY hard to see 7B2 in tissues and even on blots unless you have an enriched tissue like pituitary or adrenal. Use a radioimmunoassay!

**14 & 15:** Human C-terminus (residues 18-31 of 7B2 CT peptide) of human 7B2.

HUMAN- SPECIFIC. Not published.

**16 & 17\***: Human 7B2 CT peptide amino terminus 1-16. Requires CPB removal of basic residues to see immunoreactivity! Prefers human, but works with mouse. Good for RIA. Reference Zhu et al 1996 PNAS.

**18\*, 19**: New mouse PC2 antiserum. (YERSLQSLRKN coupled to succinylated hemocyanin) 18 most commonly used. See antisera 4 and 5 for characteristics. Reference Muller et al JCB 1997.

**23\* & 24**: C-terminus of rat/mouse 7B2 (18-31). Good for RIA. Reference 1996 Zhu PNAS.

**26\* & 27**: mouse PC2 propeptide. Antigen is His58 to Asp80 of mouse proPC2 coupled to hemocyanin. Good for RIA and immunoppt. Reference Muller et al 2000 JBC.

**35 & 36\***: Drosophila PC2 antiserum (C-terminus of Drosophila sequence). Reference paper. Works for ICC, immunoppt, Western blotting. Hwang et al, 2000 JBC. This is a good neuroendocrine marker in Drosophila. Ask us for it!

**37 & 38\***: QERAER antiserum to mouse proSAAS. Blots poorly and does not immunoppt; not good for ICC. Reference Sayah 2000 J. Neurochem.  
QERARAEAEQEAED peptide

**39 & 40\***: LENP antiserum to mouse proSAAS CT peptide. Works for immunoppt but not great for blotting of endogenous stuff (probably due to removal of CT peptide?). Not good for ICC. Reference Sayah 2000 J. Neurochem paper. LENPSPQAPA.

**\*41 & 42**: raised to ACTH 1-18. Used for immunoppt to replace antiserum JH93 of Dick Mains. Recognizes POMC, ACTH and cleaved ACTH (alpha MSH). Reference Fortenberry et al, JBC, 2002

**43 & 44**: N-terminus of mouse SAAS (ie, the SAAS peptide itself). Not good for ICC. Not published. SLAASPLVETSTPLRL used for RIA with N-terminal Y.

**45\* & 46**: Raised to recombinant His-tagged 21 kDa mouse proSAAS. Good for Western, IP and ICC. Not published yet -except by John Hutton for ICC (see his reference). No carrier.

**47, 48**: TMEM66. CQNKGWDGYDVQ is the peptide, KLH the protein. Never tested.

**49, 50**: *C. elegans* 7B2 - never tested- ask us for it! should be a great neuroendocrine marker. ESLQKILEENNMHANT is the peptide, KLH the protein

**91,92**: Polyarginine antisera (raised to D9R coupled to KLH). Not published yet. Works well in elisa (for example, in determining blood levels of administered D9R)

**93:** Proghrelin C-terminus of human protein (EEAKEAPADK) Not published. Great for Western blotting.

**94:** Ghrelin peptide C-terminus (VQQRKESKKPPAKLQP) Not published. Works for its own peptide ELISA and also for RIA.

**95,96:** Octanoylated ghrelin- a small peptide containing the Ser Octanoyl group. GSS(octanoyl)FLSPEHQ; sequence is conserved among vertebrates so works with both human and mouse. Some cross-reaction with un-octanoylated ghrelin. Works in ELISA against the octanoylated peptide. Made by Covance. 2007

**s43, s44:** Seth's human furin polyclonal (raised in rabbits at LSUHSC against our recombinant human furin). Excellent titer in elisa- 100K for #s44!); not published. We also have some human furin monoclonal from this lab (cannot find in 2012). No carrier.

**5519, 5520:** Clec3a- Raised to sequence H2N-CSFLNWDRAQPSG-amide from mouse CLEC3A coupled to hemocyanin via Cys (NEP, 2008). Never used.

**6426, 6427:** Augurin- EGPVPSKTNVAVCG Aug-1 peptide, Augurin 42-53 coupled via cysteine to hemocyanin. (NEP did this) Does not recognize cell products by blotting or immunoprecipitation. NEP made, 2008.

**MD235, 236-** Augurin- raised to full-length human His-tagged augurin. Works well for blotting full-length and cleaved recombinant product, but not cell-synthesized material; does not work for immunoprecipitation for unknown reasons. Used in Ozawa augurin paper.

**MD237,238 or 243/244** Augurin -raised to FRHGASVNYDDY (the C-terminus of human augurin 137-148) coupled to succinylated hemocyanin. Does not work for Western or immunoprecip, for unknown reasons (buried antigen? tyrosine sulfation?). Works in ELISA only against the immunogen. Covance 2010

**mPC1 -Monoclonal antibody to mPC1** (Covance, summer 2011) raised against multimeric mouse PC1/3. Not high titer; IgG needs to be purified. Several positive clones frozen in N2.

**MD275 and 276** mouse FGF23 C-terminal antiserum, CSRELPSAEEGGPAASD. Raised to the same antigen as previous C-terminal published antiserum in the fall of 2012. 276 is best; we have a production bleed. Confirmed by Western blotting using recombinant material, 14 kDa band. Note that we now know that these are likely phosphorylated segments. Maybe should treat sample with AP prior to western

**MD279, 280-** human C-terminal antiserum of FGF23, CSQELPSAEDNSPMASDP Made winter 2012. Again, this epitope contains phosphorylated proteins. Maybe should treat sample with AP prior to western

We also have a rabbit antiserum raised against a chemically phosphorylated human FGF23 peptide, CSQELP[pS]AE, conjugated to keyhole limpet hemocyanin with maleimide (Covance). See Lindberg *et al.*, J Bone Miner Res. 2015 Mar;30:449-54.

### **STEINER ANTISERA: (bequest from Dr. Donald Steiner)**

Human PC5: epitope is DYDLSHAQSTYFNDPKWPS (never published?) probably used in ghrelin paper (see below) to confirm PC5 ko.

Ghrelin antiserum (mouse; see Steiner ghrelin paper PMID:17050541). Good for tissue staining.

Obestatin antiserum (epitope unknown)

### **OLDER ENKEPHALIN ANTISERA:**

**Lucy (or 215):** raised to Leu-enkephalin coupled to thyroglobulin with glutaraldehyde. Good for RIA and Westerns. Recognizes Leu-enk and amino terminally extended forms only. Reference: Lindberg, I., and Dahl, J.L. (1981) Characterization of enkephalin release from rat striatum. J. Neurochem. 36, 506-512.

NZA- raised against Met-enk-Thyroglobulin AND met-enk-BSA

NZB- raised against Leu-enk-thyroglobulin AND leu-enk-BSA

Pat (also known as 207) raised ONLY against Leu-enk coupled to thyroglobulin

All of the above unpublished

**Cass and Dick:** raised to Met-enkephalin-RGL coupled to hemocyanin with ECDI. Good for immunoprecipitation, RIA, and Westerns, Cass used most often. Recognizes the octapeptide and amino terminally extended forms. Reference: Lindberg, I., and White, L. (1986) Reptilian enkephalins: implications for the evolution of proenkephalin. Arch. Biochem. Biophys. 245, 1-7.

**Betty:** raised to Met-enk coupled to hemocyanin with ECDI. A low titer but reasonably sensitive Met-enk antiserum for RIA. Not published.

**Xandra:** raised against Met-enk-RF coupled to succinylated hemocyanin with ECDI; recognizes proenkephalin, Peptide B and Met-enk-RF. Good for immunoprecip, RIA, and blotting. Reference: Lindberg, I., and Thomas, G. (1990) Cleavage of proenkephalin by a chromaffin granule processing enzyme. Endocrinology 126, 480-487.

**Yolanda:** raised against bovine proenkephalin Peptide B (C-terminal fragment of proenkephalin) coupled to hemocyanin with ECDI ; recognizes Peptide B, but directed against the non-Met-enk-RF portion of the molecule. Reference: Lindberg, I., and White, L. (1986) Distribution of immunoreactive Peptide B in the rat brain. Biochem. Biophys. Res. Commun. 139, 1024-1032.