# CART (55-102) (Rat, Mouse, Bovine) - Antibody for Immunohistochemistry

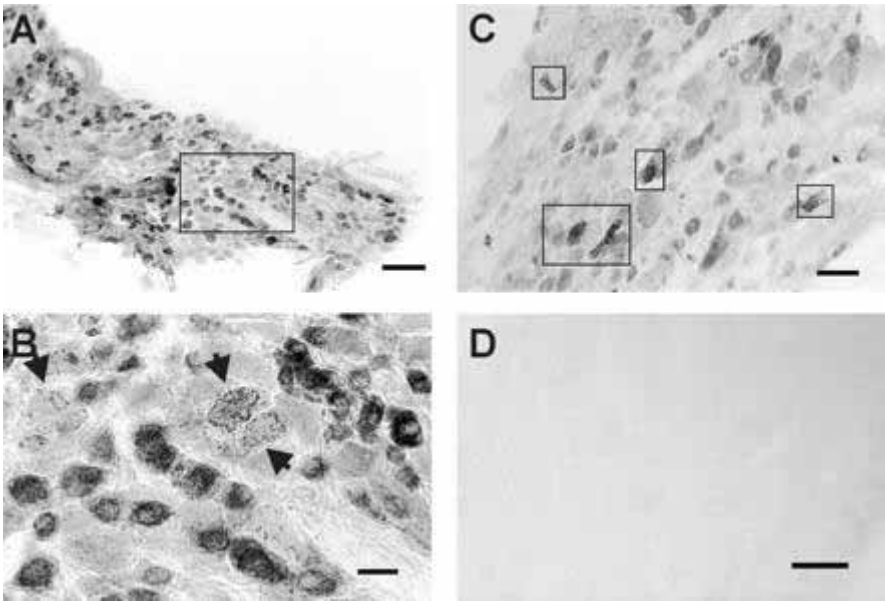
| Catalog# | Standard size | Price | Order |
|---|---|---|---|
| **H-003-62** | **50 µl** | **$450.00** | ORDER |

Product description | More information | References

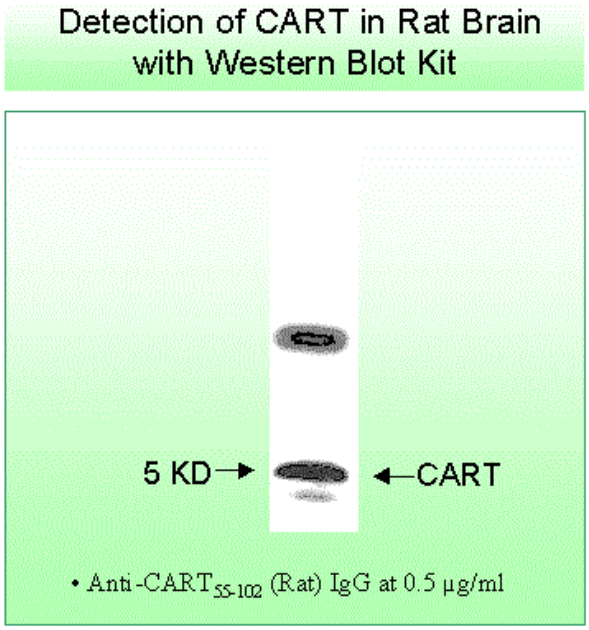
CART Preprohormone Schematic (Rat)

Prepro-CART Sequence (Rat)

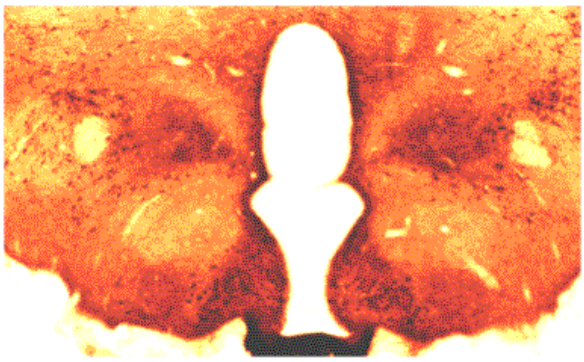
**Image**



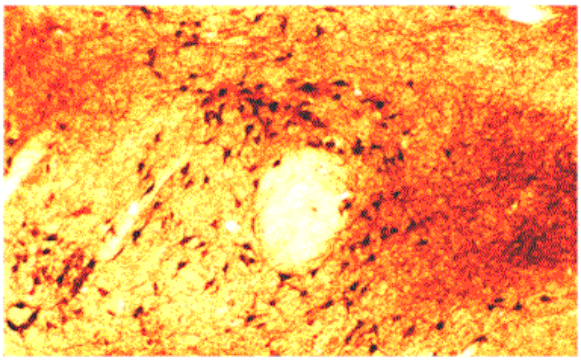

Photomicrographs of sections through rat major pelvic ganglia labeled with CART antisera or CART antisera preabsorbed with the CART peptide 55-102 using the immunoperoxidase method. A) Low-magnification view showing that numerous ganglion cells are strongly labeled. B) Higher-magnification view of an area outlined in A in which CART-LI was detected in some of the smaller-diameter ganglion cells. Some of the larger-diameter ganglion cells, which are not labeled, are invested with varicose CART-LI endings (arrows). C) A section of major pelvic ganglion showing clusters of intensely labeled, small-diameter, CART-positive cells, which are boxed in. D) A section of major pelvic ganglion processed with CART antisera preabsorbed with the peptide (10 µg/ml). Immunoreactivity is not detectable in this section. Bar = 100 µm (A and D), and 25 µm (B), and 50 µm (C) [Dun et al. Biol Reprod. 2000 Nov;63(5):1518-24.]



Rat Brain Stained with Anti-CART(55-102) (Rat) Serum
Dr. Nae J. Dun's Laboratory,
East Tennssee State University



Low Magnification



High Magnification

**Protocol for Immunohistochemistry**

Rats were anesthetized with urethane (1.2 g/kg i.p.) and intracardially perfused with 0.1 M PBS, followed by freshly prepared, 4% paraformaldehyde in PBS. The epididymis, vas deferens and major pelvic ganglia were removed, postfixed for 2 h, and immersed in 30% sucrose/PBS overnight. Tissues were sectioned to 40 µm with a Vibratome (Technical Products International, Inc., St. Louis, MO) and processed for CART-LI by the avidin-biotin complex (ABC) or fluorescent techniques, as described elsewhere [21, 22]. In addition, some sections were set aside for double-labeling experiments, in which only the fluorescent method was used [21, 22].

In the ABC method, tissues were first treated with 3% $H_2O_2$ to quench endogenous peroxidase, washed several times in Tris-buffered saline, and blocked with 10% normal goat sera (Vector Laboratories, Burlingame, CA). Tissues were incubated in the primary antibody to CART peptide fragment 55-102 (1:10 000 dilution with 0.4% Triton X-100 and 1% BSA in PBS) for 48 h at 4°C with gentle agitation. The CART antiserum, a rabbit polyclonal from Phoenix Pharmaceuticals, Inc. (Mountain View, CA), exhibits 100% cross-reactivity with the rat CART peptide 55-102 (Phoenix Pharmaceuticals). After thorough rinsing, sections were incubated with biotinylated antirabbit immunoglobulin (Ig) G (1:150 dilution; Vector Laboratories) for 2 h. Sections were rinsed with PBS and incubated in ABC solution for 1 h (1:100 dilution; Vector Laboratories). After several rinses in Tris-buffered saline, sections were developed in diaminobenzidine-$H_2O_2$ solution and washed for at least 2 h with Tris-buffered saline. Sections were mounted on slides with 0.25% gel alcohol, air-dried, dehydrated with absolute alcohol followed by xylene, and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA).

For the fluorescent method, tissues were first blocked with 10% normal goat sera and then incubated with CART antisera (1:2 000 dilution with 0.4% Triton X-100 and 1% BSA in PBS) for 48 h in a cold room with gentle agitation. After several washes with PBS, sections were incubated with biotinylated antirabbit IgG (1:50 dilution; Vector Laboratories) for 2 h. After several washes in PBS, tissues were incubated with Fluorescein Avidin D (1:50 dilution; Vector Laboratories). Lastly, tissues were washed for 30 min with PBS, mounted in Citifluor (Ted Pella, Redding, CA), and coverslipped.

In the case of double-labeling studies, the technique of sequential labeling with primary antisera from two different hosts was used . Tissues were first processed for fluorescent CART-LI as described earlier. Thereafter, tissues were washed with PBS for at least 2 h, blocked with normal horse sera, and then incubated with tyrosine hydroxylase (TH) antisera (1:500 dilution with 0.4% Triton X-100 and 1% BSA in PBS) for 48 h in a cold room with gentle agitation. The TH antiserum was a mouse monoclonal from Chemicon International, Inc. (Temecula, CA), and the specificity of the antibody has been extensively evaluated . After washing with PBS for 30 min, tissues were incubated with Avidin Texas Red (Vector Laboratories) for 4 h, washed for 30 min with PBS, mounted in Citifluor, and coverslipped. Sections were examined with a Nikon EC600 fluorescent microscope and photographed. [Dun et al. Biol Reprod. 2000 Nov;63(5):1518-24.]

| | |
|---|---|
| **Catalog #** | H-003-62 |
| **Standard Size** | 50 µl |
| **Sequence** | Ile - Pro - Ile - Tyr - Glu - Lys - Lys - Tyr - Gly - Gln - Val - Pro - Met - Cys - Asp - Ala - Gly - Glu - Gln - Cys - Ala - Val - Arg - Lys - Gly - Ala - Arg - Ile - Gly - Lys - Leu - Cys - Asp - Cys - Pro - Arg - Gly - Thr - Ser - Cys - Asn - Ser - Phe - Leu - Leu - Lys - Cys - Leu [Disulfide bonds between Cys 1 - Cys 3, Cys 2 - Cys 5, Cys 4 - Cys 6] |
| **Species** | Rat, Mouse, Bovine |
| **Host** | Rabbit |
| **Reconstitution** | For consistent and reproducible results, reconstitute with 50µl of distilled water for the equivalent of undiluted antiserum immediately before use. Reconstituted antibody can also be aliquotted (5~10ul) and stored frozen at 20°C to 70°C in a manual defrost freezer. Avoid repeated freeze-thaw cycle. |
| **Storage Condition** | For optimal results, use the antibody as soon as possible after reconstitution. Store in lyophilized form unless needed and reconstitute immediately before use. Once reconstituted, the antibody should be stable for a few days at -4°C. For storage up to a few months, prepare small aliquots after reconstitution and freeze at -20°C or -80°C. Repeated freeze thaw cycles should be strictly avoided. |
| **Content** | This vial contains 50µl of rabbit anti-CART (55-102) (Rat, Mouse, Bovine) serum in the lyophilized form. |
| **Recommended Dilution Factor** | Immunofluorescence: 1:2000<br>PAP or ABC: 1:10000 |

**Cross Reactivity**

| ***Peptide*** | ***% Cross-reactivity*** |
|---|---|
| CART 55-102 (Rat, Mouse, Bovine) | 100 |
| CART 55-102 (Human) | 100 |
| CART (61-102) (Human) | 71.83 |
| Agouti-Related Protein 83-132-NH2 (Human) | 0.01 |
| Leptin (Human) | 0 |
| Neuropeptide Y (Human, Rat) | 0 |
| Alpha-MSH | 0 |
| Orexin A (Human, Mouse, Rat) | 0 |
| Orexin B (Human) | 0 |
| MCH (Rat) | 0 |