

BOVINE BETA-CASOMORPHIN RIA KIT
125 TUBES (Cat. # BBCM42HK)

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BOVINE BETA-CASOMORPHIN RIA KIT 125 TUBES (Cat. # BBCM42HK)

I. INTENDED USE

Milk proteins are potential sources of opioid agonists and antagonists (1-3). Caseins from several species including bovine and human contain peptide fragments with opioid-like activity which interact particularly with μ -type opioid receptors in the brain (1-3). These casein derived peptides with opioid like-activity are collectively known as beta-Casomorphins of which beta-Casomorphine (1-7) and Morphiceptin (beta-Casomorphin 1-4) are predominant. Bovine beta-Casomorphins are generated as a result of milk product ingestion in the intestinal tract by gastro-intestinal enzymes (4-6). These beta-casomorphins have a role in reproductive physiology and may also play a role in whole body metabolism.

It is important to determine the regulation and/or the role of beta-Casomorphin as it relates to opioid -like activity in various patho-physiological states particularly related to reproduction.

Millipore's Bovine Beta-Casomorphin Radioimmunoassay (RIA) Kit utilizes an antibody, which specifically recognizes Bovine Beta-Casomorphin. Assay sensitivity of 15.6 pg/ml can easily be achieved when using 10 times concentrated extract from 5 ml human plasma samples subjected to C-18 Sep-Pak Cartridges extraction procedure in a two-day, disequilibrium assay. *This kit is for research purposes only.*

II. PRINCIPLES OF PROCEDURE

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 40%-50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

Millipore's Bovine Beta-Casomorphin assay utilizes ^{125}I -labeled Bovine Beta-Casomorphin and a Bovine Beta-Casomorphin antiserum to determine the level of Beta-Casomorphin in acidified human plasma after C18 Sep-Pak extraction by the double antibody/PEG technique.

III. REAGENTS SUPPLIED

Each kit is sufficient to run 125 tubes and contains the following reagents.

A. Assay Buffer

Buffer containing Human Serum Albumin and 0.08% Sodium Azide

Quantity: 50 ml

Preparation: Ready to use

B. Bovine Beta-Casomorphin Antibody

Rabbit anti-Bovine Beta-Casomorphin Serum in Assay Buffer

Quantity: 13 ml / vial

Preparation: Ready to use

C. ¹²⁵I- Bovine Beta-Casomorphin

¹²⁵I-Bovine Beta-Casomorphin Label (<1.5 μ Ci, <56 kBq)

Tracer is lyophilized for stability. Freshly iodinated label contains <1.5 μ Ci, (56 kBq), calibrated to the 1st Monday of each month.

Quantity: 13.5 ml / vial upon hydration.

Preparation: Contents Lyophilized. Hydrate with 13.5 ml of Assay Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

D. Beta-Casomorphin Standard

Recombinant lyophilized Bovine Beta-Casomorphin standard in Assay Buffer

Lyophilized for stability.

Quantity: 1 ml / vial upon hydration

Preparation: Contents Lyophilized. Hydrate with 1 ml distilled or de-ionized water. The actual concentration of bovine beta-casomorphin present in the vial will be lot-dependent. Please refer to the analysis sheet for exact concentration present in a specific lot.

E. Bovine Beta-Casomorphin Quality Controls 1 & 2

Recombinant lyophilized Bovine Beta-Casomorphin in Assay Buffer.

Lyophilized for stability.

Quantity: 1 ml/vial upon hydration

Preparation: Contents Lyophilized. Hydrate with 1 ml distilled or de-ionized water.

F. Rabbit Carrier

Diluted Rabbit Serum

Quantity: 2 ml / vial

Preparation: Ready to use

III. REAGENTS SUPPLIED (continued)

G. Precipitating Reagent

Goat anti-Rabbit IgG Serum, 3% PEG and 0.05% Triton X-100 in 0.05M

Phosphosaline, 0.025M EDTA, 0.08% Sodium Azide

Quantity: 130 ml/vial

Preparation: Ready to use; chill to 4°C.

IV. STORAGE AND STABILITY

Refrigerate all reagents between 2 and 8°C for short-term storage. For prolonged storage, (>2 weeks), freeze all the reagents at $\leq -20^{\circ}\text{C}$. Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at $\leq -20^{\circ}\text{C}$. Do not mix reagents from different kits unless they have the same lot number. Store remaining hydrated Standard, Quality Controls and Tracer at $\leq -20^{\circ}\text{C}$.

V. REAGENT PRECAUTIONS

A. Radioactive Materials

This radioactive material may be received, acquired, possessed and used only by research personnel or clinical laboratories for in vitro research tests not involving internal or external administration of the material, or the radiation there from to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of the U. S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

The following are suggested general rules for the safe use of radioactive material. The customer's Radiation Safety Officer is ultimately responsible for the safe handling and use of radioactive material.

1. Wear appropriate personal devices at all times while in areas where radioactive materials are used or stored.
2. Wear laboratory coats, disposable gloves, and other protective clothing at all times.
3. Monitor hands, shoes, and clothing and immediate area surrounding the workstation for contamination after each procedure and before leaving the area.
4. Do not eat, drink, or smoke in any area where radioactive materials are stored or used.

V. REAGENT PRECAUTIONS (continued)

5. Never pipette radioactive material by mouth.
6. Dispose of radioactive waste in accordance with NRC rules and regulations.
7. Avoid contaminating objects such as telephones, light switches, doorknobs, etc.
8. Use absorbent pads for containing and easily disposing of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform Radiation Safety Officer.

B. Sodium Azide

Sodium Azide has been added to all reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

C. Human Serum Albumin

HSA used in the preparation of this product is made from human serum and has the potential for bloodborne pathogens. Do not get in eyes, on skin, on clothing. Personal protective equipment must be worn when handling this material.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Borosilicate glass tubes, 12 x 75 mm. (NOTE: Polypropylene or polystyrene tubes may be used if the pellet formation is acceptably stable.)
2. 5 ml and 100 μ l pipettes with disposable tips
3. 10 μ l, 100 μ l & 1.0 ml repeating dispenser
4. Refrigerated swing bucket centrifuge capable of developing 2,000 – 3,000 xg. (Use of fixed-angle buckets is not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter

VII. SPECIMEN COLLECTION AND STORAGE

1. A minimum of 5 ml Human plasma should be used. After collecting blood in EDTA containing tubes, plasma is immediately separated centrifuging the blood for 10-30 min at 3000 rpm in a refrigerated centrifuge. Plasma should then be acidified with 0.1 N HCl at a concentration of 0.1 ml acid / ml plasma. Acidified plasma can then be stored at -20 C till further processing for the extraction of Beta-Casomorphin on a C18 Sep-Pak Cartridges.
2. Care must be taken when using heparin as an anticoagulant, since excess heparin may provide falsely high values. Use no more than 10 IU heparin per ml of blood collected.
3. For longer storage, specimens should be aliquot and stored at $\leq -20^{\circ}\text{C}$. Multiple freeze/thaw cycles should be avoided.
4. Avoid using samples with gross hemolysis or lipemia.

VIII. SAMPLE PREPARATION/EXTRACTION PROCEDURE

A. Reagents and Equipment required but not supplied

1. C18 Sep-Pak Plus Cartridges (820 mg) from Waters Corporation (Part # WAT023635)
2. Speed Vacuum Apparatus from Savant or some other company.
3. 5, 10 and 25 ml plastic syringes
4. Methanol
5. 1 N Hydrochloric Acid
6. Urea
7. Glacial Acetic Acid
8. Ethanol
9. Phosphate Buffered Saline (PBS) pH 7.4

All reagents should be of high purity.

VIII. SAMPLE PREPARATION/EXTRACTION PROCEDURE (continued)

B. Reagent Preparation:

1. 0.1 N HCl: Dilute 1 ml concentrated HCl (11 N) with 1100 ml Distilled Water.
2. 4% Acetic Acid-Water Solution: Dilute 40 ml of Glacial Acetic Acid with 960 ml Distilled Water.
3. 4% Acetic Acid-Ethanol Solution: Add 40 ml of Glacial Acetic Acid in 960 ml Ethanol.
4. 8 M Urea: Dissolve 484.8 gram Urea into 1000 ml final volume with Distilled Water.

C. Extraction Protocol: Before starting the extraction procedure through the C18 Sep-Pak Cartridges, dilute 5.5 ml Acidified Plasma (from Section VII of Specimen Collection and Storage) with 5 ml PBS. Centrifuge the diluted acidified plasma sample for 30 minutes at 3000 rpm in a refrigerated centrifuge). Dilution and centrifugation of acidified plasma helps in smooth passage of sample through the C18 Sep-Pak Cartridges.

1. Pass 5 ml Methanol using a plastic syringe through C18 Sep-Pak Cartridges.
2. Pass 5 ml 8 M Urea using a plastic syringe through the same C18 Sep-Pak Cartridges.
3. Pass 8 ml Water using a plastic syringe through the same C18 Sep-Pak Cartridges.
4. Pass 10.5 ml of Acidified and Diluted Plasma Sample using a plastic syringe through the same C18 Sep-Pak Cartridges. Discard the effluent.
5. Wash the same C18 Cartridges by passing through it 8 ml of 4% Acetic Acid-Water Solution. Discard the effluent.
6. Elute slowly (1-2 ml/minute) Beta-Casomorphin with 3 ml of 4% Acetic Acid-Ethanol Solution from the same C18 Sep-pak Cartridges in 12*100 mm glass test-tube. Do not discard this effluent as it contains the analyte.
7. Remove the solvent by Speed Vacuum at room temperature and/or temperature not higher than 45 °C.
8. After all the solvent is evaporated, as evident by the absence of any fluid and acetic acid smell, add 0.5 ml Assay Buffer and vortex the tube vigorously to reconstitute the analyte at 10X concentration in assay buffer.
9. Centrifuge the extracted analyte for 30 minutes at 3000 rpm in a refrigerated centrifuge and decant or aspirate the extracted analyte in a separate clean glass test tube.

Store the extracted analyte at -20°C until analyzed by Beta-Casomorphin RIA.

IX. ASSAY PROCEDURE

For optimal results, accurate pipetting and adherence to the protocol are recommended.

A. Bovine Beta-Casomorphin Standard Preparation

Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Beta-Casomorphin Standard with 1 ml distilled or de-ionized water into the glass vial to give the concentration described in the analysis sheet. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

Label six glass tubes 1, 2, 3, 4, 5 and 6. Add 0.5 ml Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 0.5 ml of the reconstituted standard to tube 1, mix well and transfer 0.5 ml of tube 1 to tube 2, mix well and transfer 0.5 ml of tube 2 to tube 3, mix well and transfer 0.5 ml of tube 3 to tube 4, mix well and transfer 0.5 ml of tube 4 to tube 5, mix well and transfer 0.5 ml of tube 5 to tube 6 and mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at -20°C . Avoid multiple freeze/thaw cycles.

Standard Concentration pg/ml	Volume of Deionized Water To Add	Volume of Standard to Add
X (Refer to analysis sheet for exact concentration)	1 ml	0

Tube #	Standard Concentration pg/ml	Volume of Assay Buffer To Add	Volume of Standard To Add
1	X/2 pg/ml	0.5 ml	0.5 ml of reconstituted standard
2	X/4 pg/ml	0.5 ml	0.5 ml of Tube 1
3	X/8 pg/ml	0.5 ml	0.5 ml of Tube 2
4	X/16 pg/ml	0.5 ml	0.5 ml of Tube 3
5	X/32 pg/ml	0.5 ml	0.5 ml of Tube 4
6	X/64 pg/ml	0.5 ml	0.5 ml of Tube 5

IX. ASSAY PROCEDURE

B. Beta-Casomorphin Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Beta-Casomorphin Quality Control 1 and Quality Control 2 with 1 ml distilled or deionized water into the glass vials. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

Note: For exact concentration of Quality Control 1 and 2, refer to Analysis Sheet. Unused portions of Quality Controls should be stored at -20°C . Avoid multiple freeze/thaw cycles.

C. Assay Set Up

Day One

1. Pipette 300 μl of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 200 μl of Assay Buffer in the Reference (B_0) tubes (5-6), 100 μl Assay Buffer in Standard tubes (7-20), Control tubes (21-24). Pipette 100 μl of Assay Buffer in sample tubes 23 through the end of the assay.
2. Pipette 100 μl of Standards (tubes 7-20) and Quality Controls (tubes 21-24).
4. Pipette 100 μl of the extracted sample (Section VIII. B.10) in duplicate.
5. Pipette 100 μl of Beta-Casomorphin Antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at 4°C .

Day Two

7. Hydrate the ^{125}I -Bovine Beta-Casomorphin tracer with 13.5 ml of Assay Buffer and gently mix. Pipette 100 μl of ^{125}I -Bovine Beta-Casomorphin to all tubes.
8. Vortex, cover and incubate overnight (22-24 hours) at 4°C .

IX. ASSAY PROCEDURE (continued)

C. Assay Set Up (continued)

Day Three

9. Add 10 μ l of Rabbit Carrier to all tubes except Total Count the tubes (1-2).
10. Add 1.0 ml of cold (4°C) Precipitating Reagent to all tubes except Total Count the tubes (1-2).
11. Vortex and incubate 20 minutes at 4°C.
12. Centrifuge, at 4°C, for 20 minutes at 2,000-3,000 xg. Note: If less than 2,000 xg is used, the time of centrifugation must be increased to obtain a firm pellet (e.g. 40 minutes). Multiple centrifuge runs within an assay must be consistent. Conversion of rpm to xg:
$$\text{xg} = (1.12 \times 10^{-5}) \text{ @ } (\text{rpm})^2$$

r = radial distance in cm (from axis of rotation to the bottom of the tube)
rpm = revolutions per minute
13. Immediately decant supernatant from all centrifuged tubes except Total Count tubes (1-2). Drain tubes for 15-60 seconds (be consistent between racks), blot excess liquid from lip of tubes and count pellet using the gamma counter according to the manufacturer's instructions.

Assay Procedure Flow Chart

Day 1					Day 2		Day 3		
Set-up	Step 1	Steps 3 - 4	Step 5	Step 6	Step 7	Step 8	Step 9	Step 10	Steps 11-13
Tube Number	Add Assay Buffer	Add Standard/QC Sample	Add Bovine Beta-Casomorphin Antibody	Vortex, Cover, and Incubate 20-24 hrs at 4°C	Add I-125 Bovine Beta-Casomorphin Tracer	Vortex, Cover and Incubate 22-24 hrs at 4°C	Add Rabbit Carrier	Add Precipitating Reagent	Incubate 20 min. at 4°C, Centrifuge at 4°C for 20 min Decant and Count
1,2	-	-	-		100 µl		-	-	
3,4	300 µl	-	-		100 µl		10 µl	1.0 ml	
5,6	200 µl	-	100 µl		100 µl		10 µl	1.0 ml	
7,8	100 µl	100 µl of tube 6	100 µl		100 µl		10 µl	1.0 ml	
9,10	100 µl	100 µl of tube 5	100 µl		100 µl		10 µl	1.0 ml	
11,12	100 µl	100 µl of tube 4	100 µl		100 µl		10 µl	1.0 ml	
13,14	100 µl	100 µl of tube 3	100 µl		100 µl		10 µl	1.0 ml	
15,16	100 µl	100 µl of tube 2	100 µl		100 µl		10 µl	1.0 ml	
17,18	100 µl	100 µl of tube 1	100 µl		100 µl		10 µl	1.0 ml	
19,20	100 µl	100 µl of reconstituted Standard	100 µl		100 µl		10 µl	1.0 ml	
21,22	100 µl	100 µl of QC 1	100 µl		100 µl		10 µl	1.0 ml	
23, 24	100 µl	100 µl of QC 2	100 µl		100 µl		10 µl	1.0 ml	
25, n	100 µl	100 µl of unknown	100 µl	100 µl	10 µl	1.0 ml			

X. CALCULATIONS

A. Explanation

The calculations for Beta-Casomorphin can be automatically performed by most gamma counters possessing data reduction capabilities or by independent treatment of the raw data using a commercially available software package. Choose weighted 4-parameter or weighted log/logit for the mathematical treatment of the data. [NOTE: Be certain the procedure used subtracts the NSB counts from each average count, except Total Counts, prior to final data reduction.]

B. Manual Calculation

1. Average duplicate counts for Total Count tubes (1-2), NSB tubes (3-4), Total Binding tubes (reference, B_0) (5-6), and all duplicate tubes for standards and samples to the end of the assay.
2. Subtract the average NSB counts from each average count (except for Total Counts). These counts are used in the following calculations.
3. Calculate the percentage of tracer bound.
$$\left(\frac{\text{Total Binding Counts}}{\text{Total Counts}}\right) \times 100$$

This should be 30-50%.
4. Calculate the percentage of total binding ($\%B/B_0$) for each standard and sample.
$$\%B/B_0 = \left(\frac{\text{Sample or Standard}}{\text{Total Binding}}\right) \times 100$$
5. Plot the $\% B/B_0$ for each standard on the y-axis and the known concentration of the standard on the x-axis using log-log graph paper.
6. Construct the reference curve by joining the points with a smooth curve.
7. Determine the pg/ml of Beta-Casomorphin in the unknown samples and controls by interpolation of the reference curve.
8. Divide all the sample values from by a factor of 10, since only 100 μ l of 10X reconstituted extract from the sample is used against 100 μ l standard.

XI. INTERPRETATION

A. Acceptance Criteria

1. The run will be considered accepted when all Quality Control values fall within the calculated Quality Control Range; if any QC's fall outside the control range, review results with the supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.

XII. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Beta-Casomorphin that can be detected by this assay is 15.6 pg/ml. However, the assay sensitivity for plasma samples is 1.56 pg/ml because of 100 µl 10X extracted analyte sample size.

B. Performance

The following parameters of assay performance are expressed as Mean \pm Standard Deviation.

$$\begin{aligned}ED_{80} &= 38.1 \\ED_{50} &= 170.6 \\ED_{20} &= 801.3\end{aligned}$$

XII. ASSAY CHARACTERISTICS (continued)

C. Specificity

The specificity (also known as selectivity) of an analytical test is its ability to selectively measure the analyte in the presence of other like components in the sample matrix.

Cross reactivity of different analytes in Beta-Casomorphin RIA

Analyte	% Cross-Reactivity
Bovine Beta-Casomorphin (1-7)	100.0
Bovine Beta-Casomorphin (1-4)	< 0.1%
Bovine Beta-Casomorphin (1-5)	< 0.1%
Bovine Beta-Casomorphin (1-8)	< 0.1%
Bovine Morphiceptin (amide)	< 0.1%
Human Beta-Casomorphin (1-4)	< 0.1%
Human Beta-Casomorphin (1-5)	< 0.1%
Human Beta-Casomorphin (1-7)	< 0.1%
Human Beta-Casomorphin (1-8)	< 0.1%
Human Beta-Casomorphin (4-9)	< 0.1%
Bovine Beta-Casomorphin (1-9)	< 0.1%
Human Beta-Endorphin	< 0.1%
Bovine Beta-Endorphin	< 0.1%
Leu-Enkephalin	< 0.1%
ACTH	< 0.1%

ND - Not detectable up to the concentration shown in parenthesis.

D. Precision

Intra and Inter Assay Variation

Sample no.	Mean pg/ml	Intra %CV	Inter %CV
1	64.17	6.22	2.61
2	300.90	5.03	6.45

Intra and inter-assays variations were performed on two samples containing low and high concentrations of Beta-Casomorphin. Data (mean and %CV) shown are from one assay with ten duplicate determinations of each sample for intra-assay precision. For inter-assay precision, data are generated using six separate assays run for the high and low samples in duplicate.

XII. ASSAY CHARACTERISTICS (continued)

E. Spike and Recovery

Beta-Casomorphin pg/ml plasma	% Expected
100	91.7 ± 5.6
1000	77.9 ± 12.8

Two different Human plasma samples were spiked with different amounts of exogenous Beta-Casomorphin. These spiked plasma samples were then subjected to extraction by C18 Sep-Pak Cartridges, the lyophilized extracts reconstituted with assay buffer (original plasma volume) and then assayed by Bovine Beta-Casomorphin RIA. Expected values are the basal levels plus the spiked amount (100 and 1000 pg/ml) of Beta-Casomorphin. The % Expected is observed value divided by expected value X 100 (Mean ± SD).

F. Linearity and Dilution

Dilution Factor	% Expected
32	72.1 ± 19.0
16	86.4 ± 1.4
8	93.6 ± 5.8
4	103.2 ± 7.2
2	88.6 ± 11.5

Two different human plasma samples were spiked with 100 and 1000 pg/ml Bovine Beta-Casomorphin, subjected to C18 Sep-Pak extraction and then serially diluted up to 4 fold for plasma samples spiked with 100 pg/ml Beta-Casomorphin and 32 fold for plasma samples spiked with 1000 pg/ml Bovine Casomorphin. The diluted extracts were then assayed by Beta-Casomorphin RIA. % Expected values (mean ± SD) are 1/32 (n=2), 1/16 (n=2), 1/8 (n=2), 1/4 (n=4) and 1/2 (n=4) dilutions of the spiked plasma extracts.

XIII. QUALITY CONTROLS

Good laboratory practice requires that quality control specimens be run with each standard curve to check the assay performance. Two levels of controls are provided for this purpose. These and any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for quality control decisions and is also responsible for making this system a written part of their laboratory manual. The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com.

Recommended batch analysis decision using two controls (Westgard Rules⁴):

1. When both controls are within ± 2 SD.
Decision: Approve batch and release analyte results.
2. When one control is outside ± 2 SD and the second control is within ± 2 SD.
Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

Technician check of systems:

1. Check for calculation errors
2. Repeat standards and controls
3. Check reagent solutions
4. Check instrument

XIV. REFERENCES

1. Teschemacher H, Koch G and Brant V (1994) Milk protein derived atypical opioid peptides and related compound with opioid antagonistic activity. In beta-Casomorphins and Related Peptides, pp 3-37 (V Brant and H Teschemacher, editors) Weinheim: VCH
2. Teschemacher H, Koch G and Brant V (1997) Milk protein derived opioid receptor ligands. Biopolymers (Peptide Science) 43, 99-117.
3. Meisel H (1997) Biochemical properties of bioactive peptides derived from milk proteins. Biopolymers (Peptide Science) 43, 119-128.
4. Svedberg J *et al* (1985) Demonstration of beta-Casomorphin immunoreactive materials in *In Vitro* Digests of bovine milk and in small intestine contents after bovine milk ingestion in adult humans. Peptides 6, 825-830.
5. Brant V *et al* (1981) Opioid activities of beta-Casomorphins. Life Sciences 28, 1903-1909.
6. Teschemacher H *et al* (1988) Human beta-Casomorphin-8 immunoreactive material in the plasma of women during pregnancy and after delivery. Regulatory Peptides 20, 107-117.

XV. REPLACEMENT REAGENTS

Reagent	Cat #
¹²⁵ I- Beta-Casomorphin (<1 .5 uCi, 56 kBq)	9042HK
Rabbit Carrier (2 ml)	RC-HK
Beta-Casomorphin Standard (1ml)	8042K
Beta-Casomorphin Antibody (13 ml)	1042HK
Precipitating Reagent (130 ml)	PR-81HK
Beta-Casomorphin Quality Control 1&2 (1 ml each)	6042K
Assay Buffer (50ml)	AB42K

XVI. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

NOTE: Appropriate license from NRC (or equivalent) must be on file at Millipore before radioactive orders can be shipped.

TELEPHONE ORDERS:

Toll Free US (866) 441-8400
(636) 441-8400

FAX ORDERS: (636) 441-8050

MAIL ORDERS:

Millipore
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.