# Ghrelin, Active

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| **Method:** | Radioimmunoassay (RIA) |
| **Kit Manufacturer:** | Millipore Research, St. Charles, MS |
| **Description:** | The Millipore Ghrelin (Active) assay utilizes 125I-labeled Ghrelin and a Ghrelin antiserum to determine the level of active Ghrelin in serum, plasma or tissue culture media by the double antibody/PEG technique. In RIA, 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 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.<br><br>Active Ghrelin is also known as circulating or acetylated Ghrelin |

## Collection and Performance Characteristics

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| **Tube type:** | Preferred: EDTA<br>Alternate: SST |
| **Minimum Volume:** | 0.5 mL (special aliquot tube)<br>a. Plasma must be acidified with fresh solutions of 50 uL of 1 N HCl and addition of 10 µl of fresh solution of Phenylmethylsulfonyl fluoride (PMSF) per one ml of plasma.<br>b. Fresh solution of PMSF in 100% methanol (or isopropanol) at a concentration of 10 mg/ml before addition to serum/plasma. (e.g. Dissolve 0.1g of PMSF in 10mL of 100% methanol or isopropanol)<br>c. Collect blood in EDTA vacutainer tube<br>d. Place in ice<br>e. Gently mix by inversion<br>f. Centrifuge at 4°C, at 1500 x g, for 10 minutes<br>g. Aliquot plasma into 2 ml Micro tubes with skirted base containing preservative, cap securely.<br>h. Store at -80°C until analysis is performed |
| **Lowest Reportable Value:** | 7.8 pg/mL |

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| **Dynamic range:** | 7.8-2000pg/mL |
| **Precision:** | Intra-assay variation is 6.5 – 9.5%<br>Inter-assay variation is 9.6-16.2% |
| **Reference Range:** | Unknown |