Brigham Research Assay Core (BRAC)
221 Longwood Avenue
Boston, MA 02115
Director: Gordon Williams, MD

Adiponectin
(total, high-, mid- and low-molecular weight multimers)

Method:	Enzyme-linked immunosorbent assay (ELISA)
Kit Manufacturer:	ALPCO Diagnostics Inc, Salem, NH
Description:	This assay operates on the principle of a "sandwich" format ELISA. The specific antibodies used in the kit are anti-human adiponectin monoclonal antibodies (MoAbs) directed to two independent epitopes. The specimens are pre-treated as described below, and total adiponectin and individual multimers of adiponectin are determined selectively, directly or indirectly. Multimers of adiponectin are classified into four fractions with this kit: 1) Total adiponectin fraction is assayed directly on the plate 2) High-molecular adiponectin fraction (equivalent of dodecamer - octodecamer): "HMW-Ad"-assayed directly on the plate 3) Middle-molecular adiponectin fraction (equivalent of hexamer): "MMW-Ad"- inferred value obtained by subtracting the concentration of HMW-Ad from the combined concentration of MMW-Ad + HMW-Ad 4) Low-molecular adiponectin fraction (equivalent of trimer including albumin-binding adiponectin): "LMWAd"- inferred value obtained by subtracting the combined concentration of MMW-Ad + HMW-Ad from the total concentration of Ad. The microtiter plate wells have been coated with an anti-human adiponectin monoclonal antibody. Adiponectin in the standards and pretreated specimens are captured by the antibody during the first incubation. Afterwards, a wash step removes all unbound material. Subsequently, an anti-human adiponectin antibody which has been biotin-labeled is added and binds to the immobilized adiponectin in the wells. After the second incubation and subsequent wash step, HRP–labeled streptavidin is added. After the third incubation and subsequent wash step, substrate solution is added. Finally, stop reagent is added after allowing the color to develop. The intensity of the color development is read by a microplate reader. The absorbance value reported by the plate reader is proportional to the concentration of adiponectin in the sample. In this kit, pre-treated normal human serum by sample pre-treatment buffer is used as the Calibrator. The sample Pre-Treatment procedure used in this assay is very important for good results. It is outlined here briefly, and explained further in the Assay.

Collection and Performance Characteristics

Tube type:	Preferred: SST Alternate: EDTA or Heparin
Minimum Volume:	0.1 mL
Lowest Reportable Value:	0.10 ug/mL
Dynamic range:	0.10- to ~24 ug/mL
Precision:	The intra-assay variations are as followed: Total: 5.0-5.4% HMW: 3.3-5.0% MMW: 10.2-13.7 LMW (calculated): 17.3-13% The inter-assay variations are as followed: Total: ~6% HMW: ~6%% MMW: ~14% LMW (calculated): ~15%
Reference Range:	unknown