

# PRODUCT INFORMATION & MANUAL

## Human Adiponectin FlowCytomix Simplex Kit

***BMS82032FF***

For research use only.  
Not for diagnostic or therapeutic procedures.



*Human Adiponectin  
FlowCytomix  
Simplex Kit*

### **North America**

#### Technical Support:

Research Products:  
888.810.6168  
858.642.2058  
tech@eBioscience.com

Clinical Products:  
877.726.8559  
858.642.2058  
tech@eBioscience.com

#### Customer Service:

888.999.1371  
858.642.2058  
info@eBioscience.com

#### Fax:

858.642.2046

### **Europe/International\***

#### Technical Support:

+43 1 796 40 40-120  
tech@eBioscience.com

#### Customer Service:

+43 1 796 40 40-304  
europe@eBioscience.com

#### Fax:

+43 1 796 40 40-400



Bender MedSystems GmbH  
Campus Vienna Biocenter 2  
1030 Vienna, Austria  
www.eBioscience.com

\* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at [www.eBioscience.com/distributors](http://www.eBioscience.com/distributors).

## TABLE OF CONTENTS

1	Reagents Provided	3
2	Intended Use	3
3	Summary	4
4	Storage Instructions – Simplex Kit	6
5	Specimen Collection and Storage Instructions	6
6	Representative Standard Curve	7
7	Performance Characteristics	8
8	Ordering Information	10

**This human Adiponectin Simplex Kit must be used in combination with FlowCytomix human Basic Kit BMS8420FF. For test procedure, measurement and calculation of results please refer to FlowCytomix human Basic Kit BMS8420FF manual.**

## **1 REAGENTS PROVIDED**

- 1 vial (175 µl) **Fluorescent Beads** (20x) coated with monoclonal antibody to human Adiponectin, Bead Population **B7**
- 2 vials human Adiponectin **Standard** (lyophilized): 1 µg/ml upon reconstitution
- 1 vial (350 µl) **Biotin-Conjugate** (20x) anti-human Adiponectin monoclonal antibody

## **2 INTENDED USE**

BMS82032FF is a bead based Analyte Detection System for quantitative detection of human Adiponectin by Flow Cytometry. **BMS82032FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

**Please note:** Samples must be **prediluted 1:250** in Assay Buffer (included in the Basic Kit BMS8420FF) before starting the test procedure.

In combination with other Simplex Kits it is recommended evaluating both, an undiluted and a 1:250 prediluted sample.

### 3 SUMMARY

Adiponectin (also referred to as Acrp30, apM1) is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. Adiponectin is exclusively secreted from adipose tissue into the bloodstream and is very abundant in plasma relative to many hormones. Levels of the hormone are inversely correlated with body mass index (BMI). The hormone plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes, obesity, atherosclerosis and non-alcoholic fatty liver disease (NAFLD).

Adiponectin was first characterised in mice as a transcript overexpressed in preadipocytes (precursors of fat cells) differentiating into adipocytes. The human homologue was identified as the most abundant transcript in adipose tissue. Contrary to expectations, despite being produced in adipose tissue, adiponectin was found to be decreased in obesity. This downregulation has not been fully explained. The gene was localised to chromosome 3p27, a region highlighted as affecting genetic susceptibility to type 2 diabetes and obesity.

Adiponectin is a 244-amino-acid-long polypeptide. There are four distinct regions of adiponectin. The first is a short signal sequence that targets the hormone for secretion outside the cell; next is a short region that varies between species; the third is a 65-amino acid region with similarity to collagenous proteins; the last is a globular domain.

Adiponectin is secreted into the bloodstream where it accounts for approximately 0.01% of all plasma protein. Plasma concentrations reveal a sexual dimorphism, with females having higher levels than males. Levels of adiponectin are reduced in diabetics compared to non-diabetics. Weight reduction significantly increases circulating levels.

Adiponectin automatically self-associates into larger structures.

Adiponectin binds to a number of receptors. So far, two receptors have been identified, with homology to G protein-coupled receptors. These have distinct tissue specificities within the body and have different affinities to the various forms of adiponectin. The receptors affect the downstream target AMP kinase, an important cellular metabolic rate control point. Expression of the receptors are correlated with insulin levels, as well as reduced in mouse models of diabetes, particularly in skeletal muscle and adipose tissue.

Adiponectin exerts some of its weight reduction effects via the brain. This is similar to the action of leptin, but the two hormones perform complementary actions, and can have additive effects.

Adiponectin affects glucose flux (gluconeogenesis, glucose uptake), lipid catabolism ( $\beta$ -oxidation, triglyceride clearance), endothelial dysfunction, insulin sensitivity and weight loss.

Administration of Adiponectin remains a promising area of research for clinical therapy in diseases such as obesity, type 2 diabetes and fatty liver disease.

For literature update refer to **[www.eBioscience.com](http://www.eBioscience.com)**

#### 4 STORAGE INSTRUCTIONS – SIMPLEX KIT

Store kit and components at 2 to 8°C. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

#### 5 SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (citrate) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible “**Hook Effect**” due to high sample concentrations (see chapter 7.4).

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human Adiponectin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

## 6 REPRESENTATIVE STANDARD CURVE

Table 1

Representative standard curve.

*Do not use this curve to derive test results. A standard curve must be run for each group of samples assayed.*

<b>Concentration (ng/ml)</b>	<b>Fluorescent Intensity (FI)</b>
50.0	158.2
16.7	55.7
5.6	19.6
1.9	7.7
0.6	3.5
0.2	2.0
0.1	1.5
0	1.2

## 7 PERFORMANCE CHARACTERISTICS

Assay performance data presented in this manual was generated in house, and is considered typical for a routine experiment in our laboratories. Each laboratory using this product should establish its own performance characteristics, and these may vary from those presented in the manual.

### 7.1 Sensitivity

The limit of detection of human Adiponectin defined as the concentration resulting in a fluorescent intensity significantly higher than that of the dilution medium (mean + 2 standard deviations) was determined to be 0.06 ng/ml.

The value shown depends on the type of flow cytometer used for analysis as well as on the respective instrument setup. The value shown is for guidance only. Optimum results for each machine can be achieved by following the instrument set up process.

### 7.2 Reproducibility

#### 7.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of human Adiponectin (high, medium high, medium low and low concentration). 2 standard curves were run on each plate. Data below show the mean intra-assay coefficient of variation for human Adiponectin (see Table 2). It has been calculated to be 2.9%.

Individual user data may vary due to differences in protein content of serum/plasma pools or individual donor serum/plasma.

Table 2

The coefficient of variation of the human Adiponectin concentration calculated for each sample.

	<b>CV Sample 1 high (%)</b>	<b>CV Sample 2 medium high (%)</b>	<b>CV Sample 3 medium low (%)</b>	<b>CV Sample 4 low (%)</b>	<b>Mean intra- assay CV (%)</b>
<b>h Adiponectin</b>	4.7	3.0	1.2	2.7	<b>2.9</b>



### 7.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of human Adiponectin (high, medium high, medium low and low concentration). 2 standard curves were run on each plate. Data below (see Table 3) show the mean inter-assay coefficient of variation for human Adiponectin, calculated on 12 determinations of each sample. It has been calculated to be 3.8 %.

Individual user data may vary due to differences in protein content of serum/plasma pools or individual donor serum/plasma.

Table 3

The coefficient of variation of the human Adiponectin concentration calculated for each sample.

	<b>CV Sample 1 high (%)</b>	<b>CV Sample 2 medium high (%)</b>	<b>CV Sample 3 medium low (%)</b>	<b>CV Sample 4 low (%)</b>	<b>Mean inter- assay CV (%)</b>
<b>h Adiponectin</b>	3.1	4.3	3.7	3.9	<b>3.8</b>

### 7.3 Specificity

Cross reactivity was tested with combinable analytes of Simplex and Multiplex Assays. There was no detectable cross reactivity observed, except for native human MPO (concentrations > 10ng/ml).

(For detailed information refer to “Combination Table“ on [www.eBioscience.com](http://www.eBioscience.com).)

### 7.4 Hook Effect

1:250 prediluted samples with expected concentrations two fold higher than the concentration of highest standard should be diluted 10 fold in Assay Buffer (1x) before assay performance to prevent false negative results due to a possible “Hook Effect”.

## 8 ORDERING INFORMATION

### North America

#### Technical Support:

Research Products:  
888.810.6168  
858.642.2058  
tech@eBioscience.com

Clinical Products:  
877.726.8559  
858.642.2058  
tech@eBioscience.com

#### Customer Service:

888.999.1371  
858.642.2058  
info@eBioscience.com

#### Fax:

858.642.2046

### Europe/International\*

#### Technical Support:

+43 1 796 40 40-120  
tech@eBioscience.com

#### Customer Service:

+43 1 796 40 40-304  
europe@eBioscience.com

#### Fax:

+43 1 796 40 40-400



Bender MedSystems GmbH  
Campus Vienna Biocenter 2  
1030 Vienna, Austria  
www.eBioscience.com

\* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at [www.eBioscience.com/distributors](http://www.eBioscience.com/distributors).