Mercodia

Total Insulin Northern Lights® MBeads Assay

Important changes made in this version

Calibrator 7 has been removed in this version, leading to several updates in the document.

Directions for Use

10-1353-01
Reagents for 500 datapoints

For Research Use Only
Not for Use in Diagnostic Procedures

Manufactured by

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

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Explanation of symbols used on labels

	Reagents for 500 datapoints
	Expiry date
	Store between 2–8°C
	Lot No.

Intended Use

Mercodia's Total Insulin Northern Lights® MBeads Assay is intended to be used for the measurement of Insulin in conditioned media from perifusion experiments or other in vitro cultures from cells derived from human and mouse specimens.

Summary and explanation of the test

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized by the ß-cells of the islets of Langerhans as preproinsulin and processed to proinsulin, which is then converted to C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids, respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain.

In the body, secretion of insulin is mainly controlled by plasma glucose concentration, and it has several important metabolic actions. Its principal function is to control the uptake and use of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis, are counteracted by hyperglycaemic hormones including glucagon, epinephrine (adrenaline), growth hormone and cortisol.

In vivo, insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Contrary, insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

In vitro, isolated pancreatic islets are studied to analyze insulin secretion rates to determine its function and activity. Such analyses allow the study of diseases (i.e., Type 1 Diabetes) in setups that can be translated later to patients or in vivo models.

Principle of the procedure

Mercodia's Total Insulin Northern Lights MBeads Assay is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. A simple washing step removes unbound enzyme-labelled antibody. The bound conjugate is detected by the reaction with the chemiluminescent substrate. A luminescence plate reader is used to read the intensity of light generated.

In the Mercodia Total Insulin Northern Lights MBeads Assay, the surface-activated magnetic agarose beads are coupled to a monoclonal mouse antibody against insulin. This antibody grants the detection of several mammalian insulins. This assay allows an increased coverage and binding, making it an ideal tool for high through-put studies to detect insulin in conditioned media derived from cells (i.e., islets isolated from human pancreas).

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- Each well can only be used once.
- Instrument settings should be optimized according to the manufacturer's instructions.
- Not for internal or external use in humans or animals.
- All samples should be handled cautiously.
- The MBeads Antibody, Control, Cal 0, 1, 2, 3, 4, 5, 6, Assay and Wash Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

  The MBeads Antibody, Control, Calibrators, Assay and Wash Buffer are labelled:

  Warning
  H317 – May cause an allergic skin reaction.
  P280 – Wear protective gloves.
  P261 – Avoid breathing vapor.
  P272 – Contaminated work clothing should not be allowed out of the workplace.
  P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
  P333 + P313 – If skin irritation or rash occurs: Get medical attention.
  P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

Material required but not provided

- Pipettes with appropriate volumes (automatic multichannel or repeating pipettes preferred for addition of MBeads Mix or other working solutions)
- Tubes, beakers, and cylinders for reagents preparation
- Vortex mixer
- Reagent reservoirs
- Microplate reader for chemiluminescence
- Microplate orbital shaker (recommended speed is 1350 cycles per minute)
- Magnetic bead-separator for 96 well plates
- Black 96 well plate

Optional:

- Microplate washing device that also supports magnetic bead washing using a biomagnetic bead-separator

Note! If other plate format is being used (i.e., 384 well plate), a suited magnet for that specific plate is required

Technical details

Automated washing

The Total Insulin Northern Lights MBeads Assay has been validated for automated washing using the "AquaMax 2000" Microplate washer (Molecular Devices). This device allows the use of the magnetic bead separator during the washing procedure.

Settings among washing devices might differ, keep in mind to adjust the settings according to the washing machine and the magnetic separator.

Example for the “AquaMax 2000” Microplate washer (Molecular Devices):

1. Aspirate (1 second, probe height 1.6 mm)
2. Dispense 280 µL (rate 5 of 5)
3. Soak 30 seconds
4. Repeat from #1 to #3 six times
5. Aspirate (1 second, probe height 1.6 mm)

Manual washing

The Total Insulin Northern Lights MBeads Assay has been validated for manual washing using a multichannel pipette. All washes were performed using the magnetic bead separator when removing the washing buffer and separating the magnet when adding the washing buffer.

Magnetic bead separator

The Total Insulin Northern Lights MBeads Assay has been validated using a magnetic beads separator (LifeSep 96F, Dexter Magnetic Technologies). When testing other plate formats (i.e., 384 well plates) a specific 384 well magnet has been tested.

Be aware of the characteristics of the magnetic separator. Not all magnets are the same, and some might allow the plate to get partially locked to the magnet. Read the instructions of your magnet carefully and test with your plates before running an experiment.

Chemiluminescent reader

The Total Insulin Northern Lights MBeads Assay has been validated using chemiluminescent readers developed by BMG Labtech (CLARIOstar and FLUOstar Omega).

Be aware of the characteristics of the reader. Follow the manufacturer´ s instructions and recommendations.

Plates
The Total Insulin Northern Lights MBeads Assay has been validated using 96 well plates produced by Greiner NBS (Greiner plate details). Other plates have been tested as well and it has been confirmed that they are compatible with Mercodia´ s Insulin Northern Lights MBeads Assay. (See table below)

Brand	Article No.	Name
Greiner Bio-One	655900	Microplate, 96 well, PS, F-bottom (chimney well), black, non-binding
Corning	3991	Corning® 96-well Black Flat Bottom Polystyrene NBS Microplate
Corning	3915	Corning® 96-well Black Flat Bottom Polystyrene Not Treated Microplate

Plate formats (96 and 384 well plates)
The current DfU specifies the protocol needed for experiments performed in a 96 well plate. Further information about the 384 well plate protocol can be found on our website **www.mercodia.com**.

Samples

The Total Insulin Northern Lights MBeads Assay reacts with Human and Mouse samples.
It is expected to react with Rat samples. (See page 15)

Perifusion samples
Media from perifusion experiments should be collected and stored properly to avoid sample degradation. It is recommended to keep the perifusate cooled after collection and store the samples at -20°C until analysis.

Conditioned media
Conditioned media from cell culture experiments should be collected and stored properly to avoid sample degradation. Centrifuge the media at 4°C to remove all possible debris and save the supernatant. It is recommended to keep the conditioned media cooled after collection and store the samples at -20°C until analysis.

Preparation of samples
No dilution is normally required, however, samples above the obtained value of Calibrator 6 should be diluted with Calibrator 0. Dilutions could be needed when working with conditioned media from hypersecreting cell cultures.

Reagents 5 X 96

Each Total Insulin Northern Lights MBeads Assay kit (10-1353-01) contains enough reagents for 500 datapoints. This would translate to 5x96 wells, **sufficient for 5x40 samples and one calibrator curve in duplicate per plate.** If needed, for larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2–8°C.

Reagent	Amount	Volume	
MBeads Antibody Mouse monoclonal anti-insulin Storage after dilution: 2–8°C for 4 weeks	1 vial	2.5 mL	Preparation, see below
Calibrators 1, 2, 3, 4, 5, 6 Recombinant human insulin Color coded yellow Concentration indicated on vial label	6 vials	1000 µL	Ready for Use
Calibrator 0 Color coded yellow	1 vial	5 mL	Ready for Use
Control Recombinant human insulin Color coded yellow Concentration indicated on vial label	1 vial	1000 µL	Ready for Use
Enzyme Conjugate 44X Peroxidase conjugated mouse monoclonal anti-insulin Storage after dilution: 2–8°C for 4 weeks	1 vial	1.0 mL	Preparation, see below
Assay Buffer Colorless solution	1 bottle	47 mL	Ready for Use
Wash Buffer 21X Storage after dilution: 2–8°C for 2 months	2 bottles	2x50 mL	Dilute 50 mL of Wash Buffer in 1000 mL of redistilled water to make Wash Buffer 1X solution
Substrate Reagent A Colorless solution *Light sensitive!* *Note! The mixture of the two components is stable for 1 day at room temperature when protected from light.*	2 bottles	2x7 mL	Mix 1:1 with Substrate Reagent B to make substrate working solution i.e., 2.5 mL + 2.5 mL
Substrate Reagent B Colorless solution *Note! Light sensitive!*	2 bottles	2x7 mL	Preparation, see Substrate Reagent A

Preparation of Enzyme Conjugate 1X solution

Prepare the needed volume of Enzyme Conjugate 1X solution by dilution of Enzyme Conjugate 44X in Assay Buffer according to the table below. Mix gently. Preferably use the Enzyme conjugate after preparation. If needed, store at 2–8°C and use within 4 weeks.

Number of wells	Enzyme Conjugate 44X	Assay Buffer
48 wells	60 µL	2.59 mL
96 wells	100 µL	4.30 mL
5x96 wells	500 µL	21.5 mL

Preparation of 0.1 % MBeads Antibody

Prepare needed volume of 0.1 % MBeads Antibody by diluting 1 % MBeads Antibody in Assay Buffer (1:10, respectively) according to the table below. Invert the vials with Assay buffer and MBeads Antibody five times. The MBeads Antibody contains beads that precipitate in the tube. The component must therefore be thoroughly mixed before use. For the same reason, pipetting must be carried out quickly and the pipette tips should not be prewetted. To make sure that all the beads are out of the pipette, pull the solution up and down 3–5 times. Mix gently. Store at 2–8°C and use within 4 weeks.

Number of wells	MBeads Antibody	Assay Buffer
48 wells	235 µL	2.115 mL
96 wells	450 µL	4.050 mL
5x96 wells	2250 µL	20.25 mL

Preparation of MBeads Mix

The mixture of Enzyme Conjugate 1X and 0.1 % MBeads Antibody is called MBeads Mix. Prepare the MBeads Mix by mixing equal volumes of Enzyme Conjugate 1X and 0.1 % MBeads Antibody (1:1) according to the table below.

Note! Start by adding Enzyme Conjugate to a tube and thereafter the 0.1% MBeads Antibody solution. Make sure that the solution is homogenous before pipetting. The MBeads Antibody 0.1% solution contains beads that precipitate in the tube. Therefore, start by adding Enzyme conjugate 1X solution and then make sure the MBeads Antibody 0.1% solution is homogenous before adding it to the mixing tube. Pipetting must be carried out quickly and the pipette tips should not be prewetted.

To make sure that all the beads are out of the pipette, pull the solution up and down 3–5 times when adding MBeads Antibody. Vortex the MBeads Mix gently or invert the tube 5 times to ensure proper mix. Store at 2–8°C and use within 2 weeks.

Number of wells	Enzyme conjugate 1X	0.1 % MBeads Antibody
48 wells	2.25 mL	2.25 mL
96 wells	4.30 mL	4.30 mL
5x96 wells	21.5 mL	21.5 mL

Preparation of Wash Buffer 1X solution

For manual washing

Prepare the needed volume of wash buffer 1X solution by dilution of Wash Buffer 21X in redistilled water (1+20) according to the table below. Mix properly. Storage after dilution: 2–8°C for 2 months.

Number of wells	Wash Buffer 21X	Redistilled water
96 wells	15 mL	300 mL
5x96 wells	2 bottles	2000 mL

For automatic washing

Prepare the needed volume of wash buffer 1X solution by dilution of Wash Buffer 21X in redistilled water (1+20) according to the table below. Mix properly. Storage after dilution: 2–8°C for 2 months.

Number of wells	Wash Buffer 21X	Redistilled water
5x96 wells	2 bottles	2000 mL

Note! When using an automatic washing machine, washing solution volumes might differ between washing machines. Priming volumes might be different between brands and models. See your washing machine specifications.

Preparation of substrate working solution

Prepare the needed volume of substrate working solution by mixing Substrate Reagent A with Substrate Reagent B (1:1) according to the table below. Mix gently and store at 2–8°C.

Note! The mixture of the two components is stable for 1 day at room temperature when protected from light.

Number of wells	Substrate Reagent A	Substrate Reagent B
48 wells	1.25 mL	1.25 mL
96 wells	2.50 mL	2.50 mL
5x96 wells	2 bottles	2 bottles

Test procedure for 96-well plate

All reagents and samples must be brought to room temperature before use.

1. Prepare wash buffer 1X solution. (See page 11)
2. Prepare the Enzyme Conjugate solution 1X (See page 10) *Light sensitive!* To avoid the Enzyme being compromised, wrap foil around the tube for protection.
3. Prepare the 0.1 % MBeads Antibody. (See page 10)
4. Pipette 5 µL each of Calibrators, controls, and samples into appropriate wells of a black 96-well assay plate using reversed pipetting.
5. Prepare MBeads Mix (See page 11) by mixing equal volumes of the prepared Enzyme Conjugate 1X solution and MBeads Antibody 0.1% solution.
6. Invert the MBeads Mix five times and pour into a reagent reservoir.
7. Use a multi-channel pipette and transfer 60 µL of MBeads Mix to the wells of the assay plate. Shake the reservoir before pipetting to ensure a homogenous solution. Hold the pipette upright so that the beads do not settle on the inside of the tips, pipetting must be carried out quickly.
8. Incubate on an orbital plate shaker for 2 hour (1350 rpm) at room temperature (18–25°C).
9. Put the assay plate on the 96-well magnetic bead separator for 1 minute.

10. Wash the plate using either automatic or manual protocol.
    a. Automatic magnetic plate washer
    Wash 6 times with 280 µL wash buffer 1X solution per well and 30 seconds soak between cycles. Adjust the settings according to the washing machine and the magnetic separator to make sure that the washer tips go down far enough to aspirate the washing solution, but not too far down so that the washer aspirates the beads. (See page 7)
    b. Manual wash
    Hold the magnet under the assay plate and discard the sample solution into the sink. Remove the magnet, and with a multichannel pipette add 280 µL of wash buffer 1X in each well. Then place the plate back on the magnet for 30 seconds. Hold the magnet under the plate and gently discard the wash into the sink.
    Repeat the procedure for a total of six wash cycles.
11. After final wash, keep the magnet under the plate and invert the plate against absorbent paper carefully. Do not tap the plate.
12. Prepare substrate working solution by mixing equal volumes of Substrate Reagent A and Substrate Reagent B. (See page 12)
13. Remove the magnet and add 25 µL substrate working solution into each well. Place plate on a shaker for approximately 5 seconds to ensure mixing.
14. Use a microplate reader for chemiluminescence. Incubate in the dark, preferably inside the reader, for 5 min at room temperature (18–25°C), without shaking.
15. Measure all visible light (glow) with an integration time of 1 second. No filter is needed. Use settings for a 96 well plate with flat bottom. Instrument settings should be used according to the manufacturer's instructions. Read within 5 minutes.
16. Apply curve fitting directly on raw data (RLU). Preferably use 5-parametric logistic regression with weighing using $1/y^2$.

Note! Be careful not to contaminate the substrate working solution with enzyme conjugate solution.

Internal controls

The Total Insulin Northern Lights MBeads Assay kit (10-1353-01) is provided with an internal control related to the standard curve samples. Other commercial controls such as controls with low, intermediate, and high insulin concentrations should routinely be assayed as samples, and results charted from day to day.

It is good laboratory practice to record the following data for each assay: kit lot number, preparation dates of kit components, RLU values for the blank, calibrators and controls.

Calculation of results

The concentration of Insulin is obtained by plotting the relative light units (RLU) of the Calibrators, except for Calibrator 0, versus their concentration. It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. It is every laboratory's responsibility to try out the functionality of the chosen curve fitting model and used software. Note that weighting of the curve fit is important to get a proper fit at the low range of the standard curve, especially when the measuring range is wide. (See page 15)

The Mercodia Total Insulin Northern Lights MBeads Assay is validated using MARS (BMG Labtech) with Five Parameter Logistic (5PL) and automatic weighting using $1/y^2$.

Note! Do not include Calibrator 0 in the curve fit.

Example of results

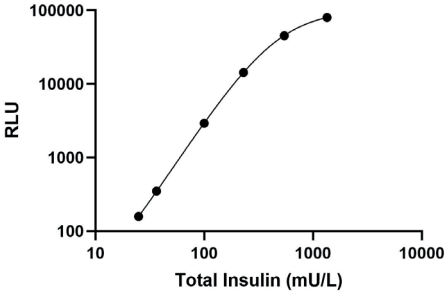
These values were obtained using BMG Labtech Clariostar with 1 s integration time, 3000 gain and 11.0 mm focal height.

Wells	Identity	RLU	Mean conc. mU/L
1 A–D	Calibrator 0	56/51/45/42	
1 E–H	Calibrator 1*	160/148/168/154	
2 A–D	Calibrator 2*	391/346/297/361	
2 E–H	Calibrator 3*	2813/2993/2682/3182	
3 A–D	Calibrator 4*	15330/14105/14623/13331	
3 E–H	Calibrator 5*	42276/40818/46554/51922	
4 A–D	Calibrator 6*	84757/84985/77386/73102	
4 E–H	Control	9491/8987/8869/9687	178.9
5 A–D	Control Low	755/735/661/722	51.3
5 E–H	Control Medium	11587/9376/11958/12483	200.1
6 A–D	Control High	45082/44395/40462/43286	516.4

*Concentration stated on vial label.

Example of calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



Specificity

The following cross reactions have been found:

C-peptide	≤ 0.02 %
Proinsulin	59 %
Rat insulin	112 %
Mouse insulin	68 %

Conversions and conversion factors

Human: 1 mU/L = 0.0435 µg/L

- For converting mU/L to µg/L, divide the value in mU/L by 23. (23 being the number of mU/L in 1 µg/L)

  1 µg/L = 23 mU/L

Mouse: 1 mU/L = 0.064 µg/L

- For converting mU/L to µg/L, divide the value in mU/L by 15.64. This number corresponds to the value acquired by multiplying 23x0.68; 23 being the number of mU/L in 1 µg/L and 0.68 the % of cross reactivity to Mouse Insulin (see cross reactivity in the section above)

Limitations of the Procedure

The assay can give higher variations at low and high concentrations of the measuring range. If your sample is in the lower range it is recommended to use a different tool (i.e., the Mercodia Insulin ELISA 10-1113-01). If your sample is in the higher range, some dilutions could be needed.

Performance characteristics

Validation of Curve Fit

The curve fitting was validated with Five Parameter Logistics with $1/y^2$ weighting.

Detection limit

Detection limit is 12.25 mU/L as determined by the methodology described in ISO11843-Part 4.

Precision and Accuracy

Samples and Control were analyzed in 4 replicates over 6 different occasions, on one kit lot, two instrument systems and on two days by two laboratory technicians.

		Coefficient of variation	
Sample	Mean value mU/L	Repeatability %*	Within laboratory %**
Sample 1	50.7	3.9	4.9
Sample 2	184.3	4.1	4.1
Sample 3	636.6	13.6	13.6
Control	174.3	5.5	6.4

*Within assay variation
**Total assay variation

Parallellism

Two human perifusion samples spiked with insulin to high concentrations within the measuring range were diluted 1/2 and 1/4. Mean recovery for parallelism is 100 % (95–104 %) with precision between samples in the dilution series ≤ 7 %.

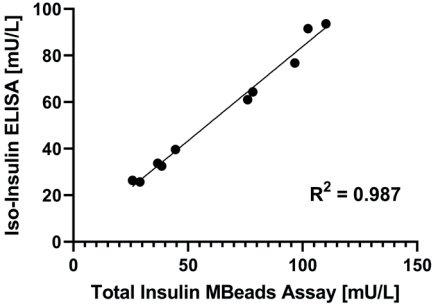
Three human perifusion samples with high insulin concentrations were diluted 1/8, 1/16 and 1/32. Mean recovery for parallelism is 101 % (95–106 %) with precision between samples in the dilution series ≤ 9 %.

Four mouse perifusion samples spiked with insulin to high concentrations within the measuring range were diluted 1/2 and 1/4. Mean recovery for parallelism is 108 % (103–111 %) with precision between samples in the dilution series ≤ 9 %.

One mouse perifusion sample with high insulin concentration was diluted 1/8, 1/16 and 1/32. Mean recovery for parallelism is 102 % (99–109 %) with precision between samples in the dilution series ≤ 5 %.

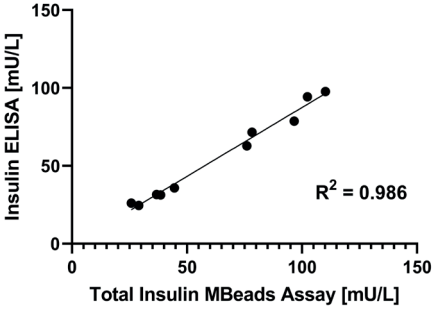
Comparison between Total Insulin Northern Lights MBeads Assay and the Iso-Insulin ELISA (10-1128-01)

Samples were analyzed and compared between methods. Results shown in the graph below, correlate the values acquired by the Total Insulin Northern Lights MBeads Assay and the Iso-Insulin ELISA. **R^2=0.987**



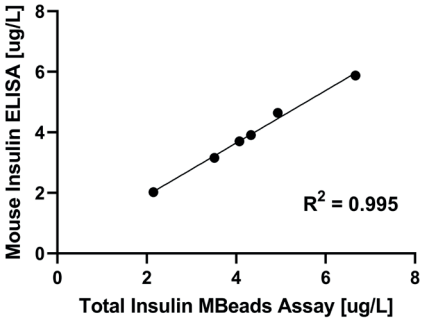
Comparison between Total Insulin Northern Lights MBeads Assay and the Human Insulin ELISA (10-1113-01)

Samples were analyzed and compared between methods. Results shown in the graph below, correlate the values acquired by the Total Insulin Northern Lights MBeads Assay and the Human Insulin ELISA. **R^2=0.986**



Comparison between Total Insulin Northern Lights MBeads Assay and the Mouse Insulin ELISA (10-1247-01)

Samples were analyzed and compared between methods. Results shown in the graph below, correlate the values acquired by the Total Insulin Northern Lights MBeads Assay and the Mouse Insulin ELISA. Results have been analyzed considering the 68% cross reactivity with mouse insulin in the Total Insulin Northern Lights MBeads Assay. **R^2=0.995**



High Dose Hook Effect

Samples with a concentration of up to 37 284 mU/L can be measured without giving falsely low results.

Calibration

Total Insulin Northern Lights MBeads Assay kit is calibrated against the 1st International Reference Preparation 66/304 for human insulin.

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: **Date:** **KIT LOT#:**

Summary of protocol sheet

Total Insulin Northern Lights MBeads Assay for 96 well plate

Prepare solutions	• Wash Buffer 1X • Enzyme Conjugate solution 1X • 0.1 % MBeads Antibody • MBeads Mix
Pipette 5 µL in each well	• Calibrators • Controls • Samples
Pipette MBeads Mix	Place 60 µL of the MBeads Mix in each well.
Incubation	2 hours / 1350 rpm / room temperature (18–25°C)
Separate	Put the plate on the 96-well magnetic bead separator for 1 minute.
Wash	• Automatic (6 times with 280 µL per well and 30 seconds soak between cycles). • Manual: Hold the magnet under the plate and discard the sample solution into the sink. Remove the magnet, and with a multichannel pipette add 280 µL of wash buffer 1X in each well. Then place the plate back on the magnet for 30 seconds. Hold the magnet under the plate and gently discard the wash into the sink. Repeat the procedure for a total of six wash cycles.
Dry up	Keep the magnet under the plate and invert the plate against absorbent paper carefully.
Prepare solution	Substrate working solutions (reagents A+B)
Add substrate	Remove the magnet and add 25 µL substrate working solution into each well. Place plate on a shaker for approximately 5 seconds to ensure mixing.
Incubate	5 minutes at room temperature protected from light.
Read chemiluminescence	1 s integration time (glow)

For full details see page 12-13

For technical support please contact: support@mercodia.com

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