Mercodia

Technical note, No: 34-0168, v.1.0

Total Insulin Northern Lights® MBeads Assay

10-1353-01

Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden
+46 18 57 00 70 | info-europe@mercodia.com | www.mercodia.com
Vat No SE-556157510001

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Alternative working protocols

1. 10 µl Sample Volume Protocol (partially validated)

The 10 µl sample volume protocol is suggested for those customers who want to use equipment that has limitations regarding smaller volumes or that are concerned about them (i.e., 5 µl). We have been able to confirm that 5 and 10 µl sample volumes give similar results (mean recovery 102%).

NOTE! Assay performance and set measurement range is only guaranteed by following the primary standardized 5 µl protocol.

Analyses

7 human samples (HP-XXX) and 7 mouse samples (MP-XXX) from perifusion experiments have been analyzed.

- Recoveries were 78-117% (mean 102%)
- CVs were 0.02-17.7%.

	Sample volume 5 µL		Sample volume 10 µL		
Sample ID	Average conc. mU/L	%CV conc	Average conc. mU/L	%CV conc	Recovery% O=10 µL, E=5 µL
HP-006	365	1,8	418	8,3	115%
HP-024	149	5,6	144	6,2	96%
HP-031	405	0,3	345	10,7	85%
HP-037	693	2,1	813	11,5	117%
HP-053	159	6,0	132	3,2	83%
HP-076	381	17,7	393	3	103%
HP-080	735	4,5	570	5,1	78%
MP-003	133	3,4	142	1,3	107%
MP-004	272	7,3	253	1,3	93%
MP-014	297	3,1	311	10	105%
MP-016	250	11,2	278	7,5	111%
MP-026	233	0,02	270	5,9	116%
MP-027	225	7,9	235	1,4	104%
MP-047	37	13,8	41	1,6	109%

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 of DfU)
2. Prepare the Enzyme Conjugate solution 1X (See page 10 of the DfU). *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 of DfU)
4. Pipette 10 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 of DfU) 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 hours (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 soak up washing solution, but not too far down so that the washer soaks up beads. (See page 7 of DfU)
    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 of DfU)
13. Remove the magnet and add 25 µL substrate working solution into each well. Place plate on an orbital 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). Use the 5-Parametric Logistic regression with weighing using 1/y2.

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

2. Low Insulin concentration Calibrator (partially validated)

The “Low Insulin concentration” calibrator is suggested for customers who want to focus on the lower end of the assay range and measure insulin concentrations below the lowest given calibrator (Calibrator 1). This can be achieved by diluting Calibrator 1 and using a sample volume of 10 µl (**protocol described in section 1 above**).

NOTE! Assay performance and set measurement range is only guaranteed by following the primary standardized 5 µl protocol.

Example of new low calibrator preparation

To prepare the lower calibrator, dilute Calibrator 1, (i.e., 24.5 mU/L to 16.3 mU/L) by adding 67 µl of Calibrator 0 and 133 µl of Calibrator 1 in a separate clean tube to gain a total volume of 200 µl of the extra calibrator. The volumes can be adjusted depending on the need.

NOTE! Calibrator concentrations might vary between lots! Calibrator concentrations are stated on the vial.

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Concentrations and conversions

Example of calibrator curve and conversion

		Human		Mouse		Rat	
Calibrators	mU/L	µg/L ng/mL	pg/mL	µg/L ng/mL	pg/mL	µg/L ng/mL	pg/mL
Extra Cal	16.3	0.71	710	1.04	1040	0.63	630
Cal 1	23	1	1000	1.47	1470	0.9	900
Cal 2	33	1.43	1430	2.11	2110	1.29	1290
Cal 3	110	4.78	4780	7.04	7040	4.29	4290
Cal 4	242	10.5	10500	15.5	15500	9.44	9440
Cal 5	527	22.9	22900	33.7	33700	20.6	20600
Cal 6	1150	50	50000	73.6	73600	44.9	44900
Cal 7	2500	108.7	108700	160	160000	97.5	97500

NOTE 1! Calibrator concentrations are stated on the vial and should be used for calculation of calibrator curve.

NOTE 2! For conversions factors see DfU (31-3198) (Human and Mouse); and TechNote 34-0166 page 12 (Rat).

Otherwise

Human: 1 mU/L = 0.0435 µg/L or 1 µg/L = 23 mU/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).

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).

Rat: 1 mU/L = 0.039 µg/L

For converting mU/L to µg/L, divide the value in mU/L by 25.76. This number corresponds to the value acquired by multiplying 23x1.12; 23 being the number of mU/L in 1 µg/L and 1.12 the % of cross reactivity to Rat Insulin (see cross reactivity in section above).