For further information refer to our website
www.miltenyibiotec.com

For technical questions, please contact your local subsidiary or distributor.

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macstec@miltenyi.com

Miltenyi Biotec

MACSPlex Mix Cytotoxic Basic Kit

For up to 100 tests

Order no. 130-125-767

Miltenyi Biotec

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140-005-643.01

Content

1. Description

This product is for research use only.

Components	0.2 mL MACSPlex Cytotoxic Detection Reagent 2, human
	2×100 mL MACSPlex Buffer
	0.35 mL MACSPlex Setup Beads
	1 MACSPlex Filter Plate
	2 adhesive foils
Size	up to 100 tests
Product format	MACSPlex Setup Beads are supplied in buffer containing stabilizer and 0.05% sodium azide. MACSPlex Buffer contains stabilizer and 0.09% sodium azide.
Storage	Store MACSPlex Setup Beads protected from light at 2–8 °C. Do not freeze. Store MACSPlex Buffer, MACSPlex Filter Plate, and adhesive foils at room temperature. The expiration dates are indicated on the labels.

140-005-643.01

1.1 Principle of MACSPlex Assays

MACSPlex Assays are designed for determining concentrations of multiple soluble analytes in a single sample. The analysis is based on MACSPlex Capture Beads, which display defined fluorescence properties and can be identified using standard flow cytometry techniques.

MACSPlex Capture Beads within a kit contain a cocktail of various fluorescently labeled bead populations, each coated with a specific antibody reacting with one of the respective analytes within the sample.

Samples containing unknown levels of analytes are incubated with the antibody-coated MACSPlex Capture Beads, and analytes bind to the specific antibody. Two detection reagents, one composed of a cocktail of biotin-conjugated antibodies specific for the analytes and one composed of Anti-Biotin-APC antibodies, are added. Consequently, sandwich complexes are formed between the MACSPlex Capture Bead, the analyte, and the detection reagent. These complexes can be analyzed based on the fluorescence characteristics of both the MACSPlex Capture Bead and the detection reagent. Standards of known quantities of given analytes are provided with the kit and are used for the quantification of the analytes within the unknown samples.

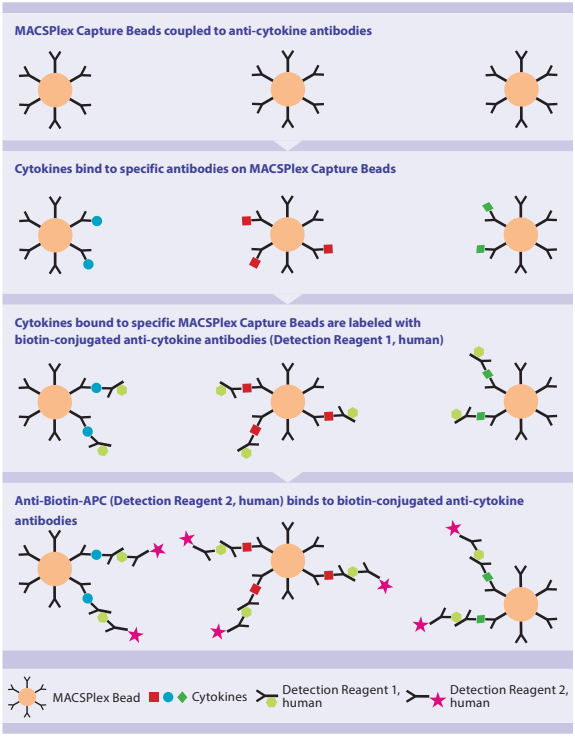


Figure 1: Principle of MACSPlex Assays.

1.2 Principle of the MACSPlex Mix Cytotoxic Basic Kit

The MACSPlex Mix Cytotoxic Basic Kit contains the supplementary components necessary to perform a MACSPlex Mix Cytotoxic Assay.

Do not mix human and mouse specificities in one experiment.

MACSPlex Mix Cytotoxic Reagents Kits are designed for detection of one or maximal up to seven analytes of choice in a single sample.

1.3 Applications

The MACSPlex Mix Cytotoxic Basic Kit in combination with the MACSPlex Mix Cytotoxic Reagents and MACSPlex Mix Cytotoxic Standard has been designed for flow cytometric detection of one or up to twelve analytes of choice in a single sample. The kit has been optimized for use with serum, plasma, and cell culture supernatants.

1.4 Reagent and instrument requirements

- MACSPlex Mix Cytotoxic Standard, human (# 130-125-766).
- MACSPlex Mix Cytotoxic Reagents, human including MACSPlex Cytotoxic Detection Reagent 1, human.
- Polypropylene or polystyrene reagent tubes for serial dilutions of the MACSPlex Mix Cytotoxic Standards as well as for preparation, dilution, and storage of unknown samples.
- Vacuum manifold or centrifuge with adapters to accommodate microtiter plates.
- Orbital shaker for microtiter plates or tubes (frequency 450–1400 rpm).

- MACSQuant® Analyzer, MACSQuant Analyzer 10 (# 130-096-343), or other flow cytometers equipped with blue (488 nm) and red (635 nm) lasers able to discriminate FITC, PE, and APC fluorescence.

  ▲ **Note:** The MACSQuant® VYB cannot be used.

- MACS® Chill 96 Rack (# 130-094-459), when using the MACSQuant Analyzer or MACSQuant Analyzer 10.

  ▲ **Note:** Store the MACS Chill 96 Rack at room temperature prior to flow cytometric acquisition to prevent the formation of condensed water on the MACS Chill 96 Rack during the acquisition.

- MACSQuant Calibration Beads (# 130-093-607), when using the MACSQuant Analyzer or MACSQuant Analyzer 10.
- (Optional) Cell culture medium, e.g., TexMACS™ Medium (# 130-097-196), DendriMACS™ GMP Medium (# 170-076-302), or RPMI 1640 supplemented with human AB serum.
- Disposable pipette tips or pipettes.
- Universal microplate lid to cover the filter plate during washing steps in the centrifuge.

2. Protocols for assay performance

▲ Use of disposable pipette tips and/or pipettes is recommended to avoid any potential contamination or cross-contamination of reagents or samples, which might invalidate test results.

▲ Avoid air bubbles.

Prepare standards and samples and transfer to MACSPlex Filter Plate	20–45 min
Add MACSPlex Mix Cytotoxic Capture Beads	2 min
Incubate plate	2 hours
Wash plate (2×)	5 min
Prepare MACSPlex Mix Cytotoxic Detection Reagent 1 and transfer to MACSPlex Filter Plate	5 min
Incubate plate	1 hour
Wash plate (2×)	5 min
Prepare MACSPlex Mix Cytotoxic Detection Reagent 2 and transfer to MACSPlex Filter Plate	5 min
Incubate plate	30 min
Wash plate (1×)	2 min
Resuspend samples with MACSPlex Buffer	3 min
Acquire data using the Express Mode of the MACSQuantify™ Software	

Figure 2: Experimental overview for the assay using a MACSPlex Filter Plate.

2.1 Preparation of the MACSPlex Mix Cytotoxic Standard

▲ Reconstitute and dilute MACSPlex Mix Cytotoxic Standard with MACSPlex Buffer, or use the same media as is used for the dilution of the unknown sample.

▲ Only use freshly prepared MACSPlex Mix Cytotoxic Standard solutions. Do not store or reuse reconstituted or diluted standards.

▲ Use polypropylene or polystyrene reagent tubes. Do not use glass vials.

For the human MACSPlex Mix Cytotoxic Assay prepare the MACSPlex Mix Cytotoxic Standard, human as described in the data sheet.

2.2 Sample preparation

▲ Handle all blood components and biological material as potentially hazardous.

▲ If unknown samples are expected or known to contain levels >2000 pg/mL, it is recommended to dilute the samples to make sure the fluorescence values are within the dynamic range of the standard curve.

▲ Use polypropylene or polystyrene reagent tubes. Do not use glass vials for sample preparation, dilution, or storage.

Preparation of serum samples

1. Allow the blood to clot for at least 30 minutes.
2. Centrifuge at 10,000×g for 10 minutes at 4 °C.
3. Transfer serum into a new tube and dilute at least 1:8 with MACSPlex Buffer, i.e., add 25 µL of the undiluted sample to 175 µL of MACSPlex Buffer.

4. Proceed to section 2.3.

Preparation of plasma samples

▲ Use freshly drawn blood samples supplemented with EDTA as anticoagulant. Do not store blood samples longer than 30 minutes before performing the assay.

1. Centrifuge peripheral blood at 10,000×g for 10 minutes at 4 °C.
2. Transfer plasma into a new tube and dilute at least 1:8 with MACSPlex Buffer, i.e., add 25 µL of the undiluted sample to 175 µL of MACSPlex Buffer.
3. Proceed to section 2.3.

Preparation of cell culture supernatant samples

1. Centrifuge cell culture supernatant at 10,000×g for 10 minutes at 4 °C.
2. Transfer the supernatant into a new tube.
3. (Optional) Dilute with cell culture medium or MACSPlex Buffer.
4. Proceed to section 2.3.

Frozen samples of serum, plasma, or cell culture supernatant

1. Thaw the samples completely and mix well by vortexing.
2. Centrifuge at 10,000×g for 10 minutes at 4 °C to remove particulates.
3. Transfer serum or plasma into a new tube and dilute at least 1:8 with MACSPlex Buffer, i.e., add 25 µL of the undiluted sample to 175 µL of MACSPlex Buffer. Cell culture supernatants can be diluted optionally with cell culture medium or MACSPlex Buffer.

4. Proceed to section 2.3.

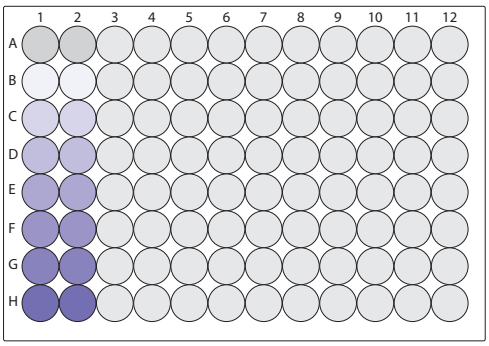
2.3 MACSPlex Mix Cytotoxic Assay

▲ Run the assay at room temperature. Work fast and keep samples protected from light, for example, cover plate or tubes with aluminum foil, especially during incubation steps.

▲ Unknown samples should be run in replicates, for example, in duplicates or triplicates and in different dilutions (e.g., undiluted, 1:3, 1:10, 1:30, 1:100) to make sure the fluorescence values are within the dynamic range of the standard curve.

2.3.1 Protocol for the assay using the MACSPlex Filter Plate

Design your assay using two columns of the MACSPlex Filter Plate for the standards. Add each of the standard samples in duplicates next to each other. Standards should be run in order from the lowest concentration (blank control: 0 pg/mL) to the highest concentration (stock solution: 10,000–50,000 pg/mL). Start with the unknown sample in the next column of the plate. For details, see figure 3.



Well position	Sample	Dilution
A1/A2	Blank control	
B1/B2	MACSPlex Mix Cytotoxic Standard (0.6 pg/mL or 3.2 pg/mL)	1:15,625 (Dilution6, 1:5^6)
C1/C2	MACSPlex Mix Cytotoxic Standard (3.2 pg/mL or 16 pg/mL)	1:3,125 (Dilution5, 1:5^5)
D1/D2	MACSPlex Mix Cytotoxic Standard (16 pg/mL or 80 pg/mL)	1:625 (Dilution4, 1:5^4)
E1/E2	MACSPlex Mix Cytotoxic Standard (80 pg/mL or 400 pg/mL)	1:125 (Dilution3, 1:5^3)
F1/F2	MACSPlex Mix Cytotoxic Standard (400 pg/mL or 2 ng/mL)	1:25 (Dilution2, 1:5^2)
G1/G2	MACSPlex Mix Cytotoxic Standard (2 ng/mL or 10 ng/mL)	1:5 (Dilution1, 1:5^1)
H1/H2	MACSPlex Mix Cytotoxic Standard (10 ng/mL or 50 ng/mL)	Stock solution (Dilution0; 1:5^0)
A3-H12	Add unknown samples	

Figure 3: Setup of the assay using a 96-well plate.

- Place the MACSPlex Filter Plate on a non-absorbent surface during loading steps and incubation, i.e. remove any tissues from the surface, to prevent the wells from running dry. Ensure that residual drops under the plate are completely removed to prevent liquid transfer, by placing the plate briefly on a tissue.
- Cover unused wells of the filter plate for later use with the adhesive foil provided with the kit.
- Washing steps are described for the use of a vaccum manifold. Alternatively, a centrifuge with an adapter for microtiter plates can be used: Put the MACSPlex Filter Plate on top of a conventional 96-flat-bottom microtiter plate with an universal lid and place both into the adapter. Centrifuge at 300×g for 3 minutes at room temperature.

- Store the MACS® Chill 96 Rack at room temperature prior to flow cytometric acquisition to prevent the formation of condensed water on the MACS Chill 96 Rack during the acquisition.

1. Pre-wet required wells of the MACSPlex Filter Plate with 200 µL of MACSPlex Buffer per well and aspirate off using a vacuum manifold designed to accommodate the filter plate (max. –300 mbar) until the wells are drained.
2. Place the filter plate briefly on a tissue to remove any residual liquid.
3. For generation of standard curve pipette 50 µL of MACSPlex Buffer or media as a blank control, 50 µL of each dilution, and the stock solution of the respective MACSPlex Mix Cytotoxic Standard to the corresponding wells of the filter plate.
4. Add 50 µL of each unknown sample per well.
5. Resuspend each MACSPlex Mix Cytotoxic Capture Beads by vortexing for at least 30 seconds.
6. Calculate the amount of required MACSPlex Mix Cytotoxic Capture Beads by determining the number of analytes and tests (standard samples and test samples) in the experiment. Add additional 2 tests to the determined test number to ensure a sufficient volume of MACSPlex Mix Cytotoxic Capture Beads.
7. Use 2 µL of each MACSPlex Mix Cytotoxic Capture Beads per test and fill up to 20 µL with MACSPlex Buffer per test.
   For example:

	MACSPlex Mix Cytotoxic Capture Beads	MACSPlex Buffer
1 analyte		
1 test	1×2 µL	18 µL
20 tests	1×40 µL	360 µL
4 analytes		
1 test	4×2 µL	12 µL
20 tests	4×40 µL	240 µL

8. Transfer 20 µL of diluted MACSPlex Mix Cytotoxic Capture Beads to each well of standard samples and tests samples.
9. Incubate filter plate for 2 hours protect from light on an orbital shaker (450 rpm).
10. Apply the filter plate to the vacuum manifold and aspirate until wells are drained. Place the filter plate briefly on a tissue to remove any residual liquid.
11. Add 200 µL MACSPlex Buffer to each well and apply the filter plate to the vacuum manifold and aspirate off until wells are drained. Place the filter plate briefly on a tissue to remove residual liquid.
12. Repeat step 11.
13. Calculate the amount of required MACSPlex Cytotoxic Detection Reagent 1, human by determining the number of analytes and tests (standard samples and test samples) in the experiment. Add additional 2 tests to the determined test number to ensure a sufficient volume of MACSPlex Cytotoxic Detection Reagent.
14. Use 2 µL of each MACSPlex Cytotoxic Detection Reagent 1, human per test and fill up to 100 µL with MACSPlex Buffer per test.
    For example:

	MACSPlex Cytotoxic Detection Reagent 1, human	MACSPlex Buffer
1 analyte		
1 test	1×2 µL	98 µL
20 tests	1×40 µL	1960 µL
4 analytes		
1 test	4×2 µL	92 µL
20 tests	4×40 µL	1840 µL

15. Transfer 100 µL of diluted MACSPlex Cytotoxic Detection Reagent 1, human to each well of standard samples and tests samples.
16. Incubate filter plate for 1 hour protect from light on an orbital shaker (450 rpm).
17. Repeat wash steps 10–12.
18. Calculate the amount of required MACSPlex Cytotoxic Detection Reagent 2, human by determining the number of tests (standard samples and test samples) in the experiment. Add additional 2 tests to the determined test number to ensure a sufficient volume of MACSPlex Cytotoxic Detection Reagent 2.
19. Use 2 µL of MACSPlex Cytotoxic Detection Reagent 2, human per test and fill up to 100 µL with MACSPlex Buffer per test.
For example:

	MACSPlex Cytotoxic Detection Reagent 2, human	MACSPlex Buffer
1 test	2 µL	98 µL
20 tests	40 µL	1960 µL

20. Transfer 100 µL of diluted MACSPlex Cytotoxic Detection Reagent 2, human to each well of standard samples and tests samples.
21. Incubate filter plate for 30 minutes protect from light on an orbital shaker (450 rpm).
22. Repeat steps 10 and 11.
23. Add 200 µL of MACSPlex Buffer to each well.
24. For sample acquisition using MACSQuant® Instruments and the Express Mode place the filter plate onto the Chill 96 Rack. To prevent liquid transfer from the wells, ensure that residual drops under the plate are completely removed by placing the plate briefly on a tissue.

▲ **Note:** Perform the flow cytometric acquisition on the same day, as prolonged storage of samples can result in increased background and reduced sensitivity.

▲ **Note:** Keep samples protected from light by using the protection lid during the flow cytometric acquisition with the MACSQuant Instrument.

2.3.2 Protocol for the assay using 1.5 mL reagent tubes

▲ Use polypropylene or polystyrene reagent tubes. Do not use glass vials.

▲ Standards should be run as duplicates. The order starts from the blank control (0 pg/mL) moving to the highest concentration (stock solution: 10,000–50,000 pg/mL).

1. Label reagent tubes for the blank control, each dilution and the stock solution of the MACSPlex Mix Cytotoxic Standard, and unknown samples.
2. Pipette 50 µL of MACSPlex Buffer or media as blank control, 50 µL of each dilution and the stock solution of the MACSPlex Mix

Cytotoxic Standard into the corresponding reagent tubes. Pipette 50 µL of each unknown sample into the corresponding reagent tube.

3. Resuspend each MACSPlex Mix Cytotoxic Capture Beads by vortexing for at least 30 seconds.
4. Calculate the amount of required MACSPlex Mix Cytotoxic Capture Beads by determining the number of analytes and tests (standard samples and test samples) in the experiment. Add additional 2 tests to the determined test number to ensure a sufficient volume of MACSPlex Mix Cytotoxic Capture Beads.
5. Use 2 µL of each MACSPlex Mix Cytotoxic Capture Beads per test and fill up to 20 µL with MACSPlex Buffer per test.
For example:

	MACSPlex Mix Cytotoxic Capture Beads	MACSPlex Buffer
1 analyte		
1 test	1×2 µL	18 µL
20 tests	1×40 µL	360 µL
4 analytes		
1 test	4×2 µL	12 µL
20 tests	4×40 µL	240 µL

6. Transfer 20 µL of diluted MACSPlex Mix Cytotoxic Capture Beads to each tube of standard samples and tests samples.
7. Incubate for 2 hours protect from light on an orbital shaker (1400 rpm).
8. Add 0.5 mL of MACSPlex Buffer to each tube.
9. Centrifuge at 3000×g for 5 minutes.
10. Carefully aspirate off the supernatant using fresh pipette tips to

avoid carry-over of samples. Leave 20 µL in the tube.

11. Resuspend the MACSPlex Mix Cytotoxic Capture Bead pellet in each tube by adding 0.5 mL of MACSPlex Buffer and pipetting up and down.
12. Repeat steps 9 and 10.
13. Calculate the amount of required MACSPlex Cytotoxic Detection Reagent 1, human by determining the number of analytes and tests (standard samples and test samples) in the experiment. Add additional 2 tests to the determined test number to ensure a sufficient volume of MACSPlex Cytotoxic Detection Reagent.
14. Use 2 µL of MACSPlex Cytotoxic Detection Reagent 1, human per test and add to the remaining 20 µL of supernatant of step 10, fill up to 100 µL with MACSPlex Buffer per test.
For example:

	MACSPlex Cytotoxic Detection Reagent 1, human	MACSPlex Buffer
1 analyte		
1 test	1×2 µL	78 µL
20 tests	1×40 µL	1560 µL
4 analytes		
1 test	4×2 µL	72 µL
20 tests	4×40 µL	1440 µL

15. Transfer 80 µL of diluted MACSPlex Cytotoxic Detection Reagent 1, human to the remaining 20 µL of supernatant in each tube of standard samples and tests samples.
16. Incubate for 1 hour protected from light on an orbital shaker (1400 rpm).

17. Add 0.5 mL of MACSPlex Buffer to each tube.
18. Centrifuge at 3000×g for 5 minutes.
19. Carefully aspirate off the supernatant using fresh pipette tips to avoid carry-over of samples. Leave 20 µL in the tube.
20. Resuspend each pellet in 0.5 mL of MACSPlex Buffer by pipetting up and down.
21. Repeat steps 18 and 19.
22. Use 2 µL of MACSPlex Cytotoxic Detection Reagent 2, human per test and add to the remaining 20 µL of supernatant of step 19, fill up to 100 µL with MACSPlex Buffer per test.
For example:

	MACSPlex Cytotoxic Detection Reagent 2, human	MACSPlex Buffer
1 analyte		
1 test	1×2 µL	78 µL
20 tests	1×40 µL	1560 µL
4 analytes		
1 test	4×2 µL	72 µL
20 tests	4×40 µL	1440 µL

23. Transfer 80 µL of diluted MACSPlex Cytotoxic Detection Reagent 2, human to the remaining 20 µL of supernatant in each tube of standard samples and tests samples.
24. Incubate for 30 minutes protected from light on an orbital shaker (1400 rpm).
25. Add 0.5 mL of MACSPlex Buffer to each tube.
26. Repeat steps 18 and 19.

27. Resuspend the pellet in each tube with 200 µL of MACSPlex Buffer
28. For sample acquisition with the MACSQuant® Express Mode, transfer samples to the MACSPlex Filter Plate. Place the MACSPlex Filter on a non-absorbent surface during loading steps, i.e., remove any tissues from the surface, to prevent the wells from running dry. Place the filter plate onto a Chill 96 Rack and measure.

▲ **Note:** Acquire cytokine standards first, beginning with the standard samples of the first dilution series in order from the blank control to the highest concentration. Then process the standard samples of the second dilution series in the same order (see figure 3). Afterwards acquire the unknown samples.

▲ **Note:** Perform the flow cytometric acquisition on the same day, as prolonged storage of samples can result in increased background and reduced sensitivity.

▲ **Note:** Keep samples protected from light by using the protection lid during the flow cytometric acquisition with the MACSQuant Instrument.

3. Flow cytometer set up

The kit includes MACSPlex Setup Beads for flow cytometer set up. MACSPlex Setup Beads are not required when using the MACSQuant® Analyzer or MACSQuant Analyzer 10 but for all other instruments. The kit is not suitable for use with the MACSQuant VYB.

3.1 Setup of the MACSQuant Instrument

Calibrate the MACSQuant® Instrument using MACSQuant Calibration Beads (# 130-093-607). For details, refer to the data sheet of the MACSQuant Calibration Beads.

After successful completing the calibration, the MACSQuant Instrument is ready for measurement. No further steps are required as all necessary setup steps are performed automatically during calibration.

3.2 Setup of other flow cytometers

The analysis of MACSPlex Mix Cytotoxic Assays requires a flow cytometer with a blue (e.g. 488 nm) and a red (e.g. 635 nm) laser, which are capable of detecting FITC, PE, and APC. For the purpose of setting up these cytometers, MACSPlex Setup Beads are included in the kit. For instructions on the setup procedures of other flow cytometers, please refer to the application note "General instructions for MACSPlex Mix Cytotoxic Kits" available on the product's webpage at www.miltenyibiotec.com/130-109-701.

4. Flow cytometric acquisition and data analysis using the MACSQuant® Express Mode

To perform the acquisition and data analysis of the MACSPlex Custom Cytokine Assay with the MACSQuant® Instrument it is recommended to use the Express Modes "MACSPlex_Standard" and "MACSPlex_Sample" to achieve automated measurement and data analysis.

For details refer to the special protocol “Data acquisition and analysis of MACSPlex Cytokine Kits using the MACSQuant Analyzer Express Modes” available at www.miltenyibiotec.com/130-125-767 under the Library tab. The minimum version number of the Express Mode package needed to run the assay on the MACSQuant Instrument is installExpressModes_213.2.20284 with MACSQuantify™ Software version 2.13.1. or higher.

To check the version number of your Express Mode package available on your MACSQuant Instrument please select **Help** > **Info** within the MACSQuantify Software (refer to figure 4). The version number of the Express Mode package is increasing with each Express Mode update. Make sure the MACSQuant Instrument contains an Express Mode package with at least the same or higher version number than the special protocol is marked with.

Overview

Relevant parameter for Express Mode experiments	human
Maximum number of standard positions (including blank) within the serial standard dilution	8
Dilution factor of serial standard dilution	5

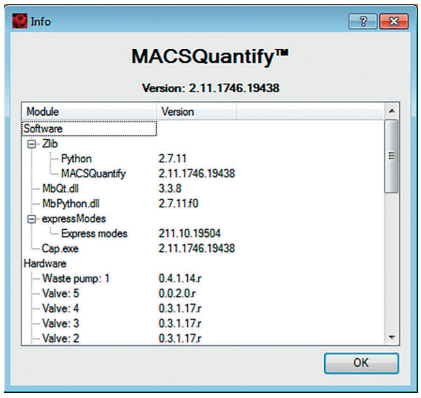


Figure 4: Identification of version number of Express Mode package (exemplary picture. Actual software and Express Mode package version may vary).

5. Troubleshooting

The following section offers solutions for problems that might be encountered when using MACSPlex Assays.

- **Variation between replicate samples:**
  MACSPlex Mix Cytotoxic Capture Beads can aggregate. Mix MACSPlex Mix Cytotoxic Capture Beads at least 30 seconds before pipetting.
- **Low bead number in samples:**
  Mix MACSPlex Mix Cytotoxic Capture Beads sufficiently before pipetting.
  Ensure that the instrument is calibrated for the relevant 96-well plate to avoid aspiration of air.
  Avoid aspiration of beads during washing steps. Do not wash or resuspend beads in volumes higher than recommended.
- **High background:**
  Avoid cross-well contamination. Ensure pipetting with multichannel pipettes and avoid touching reagent in the plate.
  The sample may be too concentrated. Test various sample dilutions.
  The background may be due to non-specific binding. Increase number of washes to remove excess of MACSPlex Cytotoxic Detection Reagent.
- **Little or no detection of cytokine in sample:**
  Sample may be too dilute. Test various sample dilutions. Use positive and negative control samples.

- **MACSPlex Mix Cytotoxic Standard Samples show low fluorescence or result in poor standard curves:**
  Check that all kit components are prepared and stored properly. Use a new vial of MACSPlex Mix Cytotoxic Standard for each experiment. Adhere to incubation times as indicated in the protocol.
  Assay was exposed to light. Keep the plate and beads covered with aluminum foil or a dark lid during all incubation steps. Use the protection lid during the sample acquisition with the MACSQuant® Instrument.
- **All samples are positive or above the high standard mean fluorescence value:**
  The samples may be too concentrated. Dilute the samples further.
- **Beads not in region or gate:**
  Ensure proper calibration of the MACSQuant Instrument. Samples containing organic solvents or samples of high viscosity should be diluted or dialyzed, respectively
- **Signal for whole plate is same as background:**
  Incorrect amount or no MACSPlex Cytotoxic Detection Reagent was added.
- **Low signal for standard curve:**
  Incubation steps were done at inappropriate temperatures, timings, or agitation.
- **Signals too high, standard curves are saturated:**
  Incubation time was too long. Adhere to incubation times as indicated in the protocol.

- **Sample readings are out of range – if below detection limit:**
  Samples may be too diluted. Adjust dilutions accordingly.
  Samples may contain no analyte. Analyte concentration may be below the detection limit. It may be possible to use higher sample volumes. Please contact Milteny Biotec's Technical Support for appropriate protocol modifications.

  If above detection limit:
  Samples contain analyte concentrations above 2 ng/mL or 10 ng/mL. Dilute the samples further.
- **High variation in samples and/or standards:**
  Multichannel pipette may not be calibrated.
  Plate washing was not uniform.
  Samples may have contained high particulate matter or other interfering substances.
  Plate agitation was insufficient.
  Cross-well contamination. Ensure pipetting with multichannel pipettes and avoid touching reagent in the plate. Change pipette tips for each well when touching the reagent.
- **Filter plate will not vacuum:**
  Vacuum pressure is insufficient. Increase vacuum pressure.
  Samples contain insoluble particles. Centrifuge samples just prior to performing the assay and use supernatant.
  High lipid concentration. Centrifuge samples, remove lipid layer, and use supernatant to perform the assay.

- **Plate leakage:**
  Vacuum pressure is too high. Adjust vacuum pressure to maximal –300 mbar.
  Place the MACSPlex Filter Plate on a non-absorbent surface during filling steps and incubation, i.e., remove any tissues from the surface, to prevent the wells from running dry.
  Ensure that residual drops under the plate are completely removed to prevent leakiness of the wells, by placing the plate briefly on a tissue, after each washing step.
  Avoid touching the plate filter with the tip of the pipette when adding reagents to the wells.

Warnings
Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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