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Not for use in diagnostic procedures.

MIA ELISA

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Photometric enzyme-linked immunosorbent assay (ELISA) for the quantitative *in vitro* determination of human Melanoma Inhibitory Activity (MIA) in streptavidin-coated microplates

Cat. No. 11 976 826 001 1 kit
96 tests

Store the kit at +2 to +8°C.

1. General Information ... 3
1.1. Contents ... 3
1.2. Storage and Stability ... 4
Storage Conditions (Product) ... 4
1.3. Additional Equipment and Reagent required ... 4
1.4. Application ... 4
1.5. Preparation Time ... 4
Assay Time ... 4

2. How to Use this Product ... 5
2.1. Before you Begin ... 5
Sample Materials ... 5
General Considerations ... 5
Precautions ... 5
Adaption for automation ... 5
Safety Information ... 5
Laboratory procedures ... 5
Waste handling ... 5
Working Solution ... 6
2.2. Protocols ... 7
Sample preparation for blood ... 7
Microplate pipetting scheme ... 7
ELISA assay ... 8
2.3. Parameters ... 9
AccuracyDetection range ... 9
Precision ... 9
Specificity ... 9

3. Results ... 10
Plotting the standard curve ... 10
Determination of the analyte concentration ... 10
Handling very concentrated samples ... 11
Typical results ... 11

4. Troubleshooting ... 12

5. Additional Information on this Product ... 13
5.1. Test Principle ... 13
Test principle ... 13
How this product works ... 13
Background information ... 13
5.2. Quality Control ... 13

6. Supplementary Information ... 14
6.1. Conventions ... 14
6.2. Changes to previous version ... 14
6.3. Trademarks ... 15
6.4. License Disclaimer ... 15
6.5. Regulatory Disclaimer ... 15
6.6. Safety Data Sheet ... 15
6.7. Contact and Support ... 15

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	blue	MIA ELISA, Anti-MIA-biotin	▪ Monoclonal antibody from mouse (clone 2.F7.3B1) ▪ Antibody binds to the native conformation of natural and recombinant human MIA. ▪ Capture antibody ▪ White lyophilizate	1 bottle
2	red	MIA ELISA, Anti-MIA-POD	▪ Monoclonal antibody from mouse (clone 1.A12.9A1), Fab-fragment conjugated with peroxidase. ▪ Antibody binds to the native conformation of natural and recombinant human MIA. ▪ Detection antibody ▪ White lyophilizate	1 bottle
3a to 3f	orange	MIA ELISA, MIA Standard	▪ Contains 0 to approximately 50 ng/ml MIAin a serum analogue matrix containing BSA, buffer, and preservatives. ⚠ ***See lot-specific label for exact content in ng/ml.*** ▪ White lyophilizate	1 bottle for each standard
4	violet	MIA ELISA, MIA control serum	▪ Contains approximately 25 ng/ml MIAin human serum. ⚠ ***See lot-specific label for exact content in ng/ml.*** ▪ White lyophilizate ▪ Positive control	1 bottle
5	red	MIA ELISA, Incubation buffer	▪ Ready-to-use solution. ▪ Clear solution with possible foaming. ▪ Dilution buffer	1 bottle, 100 ml
6	white	MIA ELISA, Washing buffer tablets	White tablets	1 bottle, 2 tablets
7	black	MIA ELISA, ABTS substrate solution	▪ Ready-to-use solution. ▪ Clear solution, slightly green.	1 bottle, 27 ml
8	foil bag	MIA ELISA, Microplate	▪ Precoated with streptavidin. ▪ Shrink-wrapped with a desiccant capsule (12 × 8 wells).	1 strip frame, 12 modules of 8 wells each
9	–	MIA ELISA, Self-adhesive Plate Cover Foil	▪ Prevents evaporation. ⚠ ***Cover the Microplate modules with the Cover Foils during each incubation step.***	2 foils

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	Anti-MIA-biotin	Store at +2 to +8°C.
2	red	Anti-MIA-POD	
3a to 3f	orange	MIA Standard	
4	violet	MIA control serum	
5	red	Incubation buffer	
6	white	Washing buffer tablets	
7	black	ABTS substrate solution	
8	foil bag	Microplate	
9	–	Self-adhesive Plate Cover Foil	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Microplate washer
- Microplate reader
- Microplate shaker

  If no shaker is used, signal levels will be considerably lower (approximately 40%) and may vary.
- Pipettes carefully calibrated
- Sterile, aerosol-resistant pipette tips
- Sterile cups for preparing dilutions
- Absorbent, disposable towels for washing steps

Data analysis

- ELISA reader: The green color of the substrate ABTS can be easily detected by eye; for numeric values however, a photometric measurement is required.
- Calculation software recommended

Adaptation to automation

The MIA ELISA can be used in automated microplate systems. The reagents are provided in excess (≥20%) for this purpose. With normal use, this is sufficient to perform the test in one cycle on most machines.

For the preparation of kit working solutions

- Double-distilled water

All reagents necessary to perform the assay are supplied with this kit.

1.4. Application

The MIA ELISA is intended for use in research studies as a method for the quantitative *in vitro* determination of MIA protein in serum and plasma within streptavidin-coated microplates.

1.5. Preparation Time

Assay Time

The assay time is 2 hours.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The MIA ELISA can be used with the following sample materials:
- Serum
- Plasma, treated with EDTA, citrate, or heparin.

⚠ ***Do use samples which are clotted, grossly hemolyzed, lipemic, or microbially contaminated.***

⚠ ***Samples must not contain sodium azide or sulfide reducing agents, such as 2-mercaptoethanol and DTT because they will interfere with peroxidase activity of the detection antibody.***

General Considerations

Precautions

- Pipette thoroughly to ensure accurate transfer of the small volumes.
- Perform a separate calibration curve simultaneously with each test series.
- Perform all measurements in duplicates.

  ℹ *All reagents necessary to perform the assay are supplied with this kit.*
- Reagents and Microplate modules of different lots must not be used in one test series.
- Equilibrate all reagents to +15 to +25°C before use.
- The ABTS substrate solution is very sensitive to contamination, therefore do not pipette directly from the bottle, instead transfer the required quantity into a separate vial.

Adaption for automation

The MIA ELISA can be used in automated microplate systems. The reagents are provided in excess (≥20%) for this purpose. With normal use, this is sufficient to perform the test in one cycle on most machines.

Safety Information

The blood of the donors for the control and standards was tested for the presence of HBsAg and antibodies to HIV-1, HIV-2, HCV, and found to be negative, according to the current quality control procedures. Since the danger of infection cannot be completely excluded, the positive control and standards must be handled with the same care as infected material. In the case of exposure, the guidelines of the appropriate health authorities must be followed.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Content	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
1	Anti-MIA-biotin (Bottle 1)	▪ Reconstitute the lyophilizate in 700 µl double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a clear, colorless solution. ⚠ ***Do not vortex.***	Store in aliquots for 6 months at −15 to −25°C to −70°C.	Immunoreagent
2	Anti-MIA-POD (Bottle 2)	▪ Reconstitute the lyophilizate in 700 µl double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a clear, slightly yellow solution. ⚠ ***Do not vortex.***		
3a to 3f	MIA Standard (Bottles 3a to 3f)	▪ Reconstitute the lyophilizate in 500 µl double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a clear, colorless solution. ⚠ ***Do not vortex.***	▪ Store 1 day at +2 to +8°C, or ▪ Store in aliquots for 6 months at −15 to −25°C to -70°C.	Step 1
4	MIA control serum (Bottle 4)	▪ Reconstitute the lyophilizate in 300 µl double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a slightly opalescent and yellow solution. ⚠ ***Do not vortex.***		
6	Washing buffer tablets (Bottle 6)	▪ Dissolve one tablet in 2 liters of double-distilled water. ▪ This results in a clear, colorless solution.	Store 1 month at +2 to +8°C.	Step 3
–	Immunoreagent	For 8 wells (1.8 ml): ▪ Add 50 µl reconstituted Anti-MIA-POD (Solution 2) to 1.7 ml Incubation buffer (Bottle 5); mix thoroughly. ▪ Add 50 µl of Anti-MIA-biotin (Solution 1); mix thoroughly. ▪ This results in a clear, colorless solution. ⚠ ***Do not vortex.***	Store 8 hours at +2 to +8°C.	Step 2

⚠ ***All lyophilizates should become clear solutions after reconstitution except the positive control. Any particles within the reconstituted solution should be considered as deterioration. The immunoreagent, substrate solution, and washing buffer should be clear and colorless. Precipitates or cloudiness in the reagent solutions should be considered as indications of instability or deterioration.***

2.2. Protocols

Sample preparation for blood

1. After bleeding, prepare serum or plasma according to standard procedures.
   ⚠ ***Do not add sodium azide or sulfide reducing agents.***

2. Suspended matter contained in the samples should be pelleted for 5 minutes by centrifugation at 13,000 × *g* prior to the assay.

3. After shock freezing, store samples at −60°C or below for up to one year.

   ⚠ ***Avoid repeated freezing and thawing.***

Microplate pipetting scheme

Set up the pipetting scheme according to the following table.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bl	Bl	P1	P1	–	–	–	–	–	–	–	–
B	Sa	Sa	–	–	–	–	–	–	–	–	–	–
C	Sb	Sb	–	–	–	–	–	–	–	–	–	-
D	Sc	Sc	–	–	–	–	–	–	–	–	–	–
E	Sd	Sd	–	–	–	–	–	–	–	–	–	–
F	Se	Se	–	–	–	–	–	–	–	–	–	–
G	Sf	Sf	–	–	–	–	–	–	–	–	–	–
H	P0	P0	–	–	–	–	–	–	–	–	P40	P40

Bl = blank (substrate solution only)
Sa to Sf = MIA Standards
P0 = positive control (MIA control serum)
P1 to P40 = samples 1 to 40
ⓘ *Perform a separate calibration curve simultaneously with each test series.*
ⓘ *Perform all measurements in duplicates.*

ELISA assay

Follow these steps to determine the amount of MIA in blood or plasma samples.

⚠ ***Equilibrate reagents to +15 to + 25°C before starting the assay. Reagents from kits with different lot numbers must not be used in one assay series.***

ⓘ *Perform all incubation steps at +15 to +25°C. Always use the same procedure to minimize inter-assay variances.*

1. Pipette 20 µl of MIA Standards (Solutions 3a to 3f), or the MIA control serum (Solution 4), or the sample material (serum or plasma).

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2. Add 180 µl of the Immunoreagent.
   – Cover the Microplate modules with the Cover Foil and incubate for 90 minutes under constant shaking at 450 rpm.

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3. Remove the solution by aspirating away the buffer.
   ⓘ *Alternatively, the Microplate may be inverted and tapped gently on a paper towel.*
   – Rinse wells 3 times with 300 µl of Washing buffer (Solution 6) for 1 minute each.

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4. Carefully remove Washing buffer by aspirating or tapping.

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5. Pipette 200 µl of ABTS substrate solution (Bottle 7) into each well.
   ⚠ ***This reagent is very sensitive to contamination. Do not pipette directly from the ABTS substrate bottle; transfer the required quantity into a separate tube.***
   – Incubate at +15 to +25°C until color development is sufficient for photometric detection, approximately 10 to 20 minutes.
   – Shake Microplates at 450 rpm during incubation with substrate solution.

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6. Perform photometric measurement at 405 nm (reference wavelength 495 nm).

2.3. Parameters

Accuracy

Characteristic	Data
Calibration	Calibrated against a master lot preparation of highly purified recombinant human MIA.
Dilution test	Target ± 8%
Recovery relative to serum	Serum 100%
	EDTA-plasma 98 ± 9%
	Heparin-plasma 94 ± 12%
	Citrate-plasma 97 ± 9%
High dosage hook effect	No hook effect was observed applying up to 1 µg/ml MIA.
Interferences	No interferences with other serum components known.
	No interference with human anti-mouse antibodies (HAMA) due to special additives.

Detection range

0.49 to 50 ng/ml
The measuring range covers the physiological as well as the pathological concentration range.
Definition: The measuring range is between the limit of quantification (≥0.49 ng MIA/ml), and the highest standard (Bottle 3f, approximately 50 ng MIA/ml).

Precision

Characteristic	Data
Sensitivity, Limit of Detection (LOD)	≥0.32 ng/ml MIA. Definition: The LOD is the lowest concentration of an analyte that the analytical process can reliably differentiate from background levels. It is defined as the absorbance mean of 21 replicates of the zero standard plus threefold the standard deviation reading from the standard curve.
Sensitivity, Limit of Quantification (LOQ)	≥0.49 ng/ml MIA. Definition: The LOQ is the lowest concentration of an analyte that can be measured with a stated level of confidence. It is defined as the minimum detectable concentration of MIA in 5 replicates showing a coefficient of variation <15% and where the 3 SD range does not overlap to 3 SD of the 0-standard. This value is higher than the LOD.
Intra-assay variance	3.8% (low sample), 4.0% (medium sample), 3.8% (high sample)
Inter-assay variance	6.5% (low sample), 6.6% (medium sample), 5.5% (high sample)
Linearity	r ≥0.99

Specificity

The MIA ELISA detects natural and recombinant human melanoma inhibitory activity protein. No cross-reaction with other serum components has been found.

3. Results

Plotting the standard curve

Use the standards provided in the kit to prepare a six point calibration curve.

1. Correct each absorbance value of all standards by subtracting the value of the reagent blank (Bl = only substrate).

2. Calculate the mean absorbance value for each standard from the duplicates.

3. Prepare a plot correlating the mean absorbance values of the standards to the analyte concentrations of the standards.
   The lot-specific concentration of each standard is listed on its bottle label.

4. Prepare the plot using either of two methods:

Method	Steps
Automatic	Enter the absorbance values and the analyte concentrations into a suitable data analysis software program. *To achieve best results, make sure the software is able to calculate the standard curve with a four-parameter Rodbard-function. Several data analysis programs perform statistical analysis, such as recalculation, mean, SD, CV, CV regression analysis on the absorbance values entered.*
Manual	Plot on semilogarithmic graph paper, the mean absorbance values on the Y-axis against the analyte concentrations on the X-axis.

Determination of the analyte concentration

Follow these steps to determine the analyte concentration:

1. Correct each absorbance value of all samples and controls by subtracting the value of the reagent blank (Bl= only substrate).

2. Calculate the mean absorbance value for each sample from the values of the duplicates.
   If the absorbance of the sample duplicates are above the absorbance of the highest standard, do not use those duplicates to determine analyte concentration in the diluted sample (next step). Repeat the assay for that sample as outlined in the next section, **Handling very concentrated samples**.

3. Determine the analyte concentration in the diluted sample using either of two methods:

Method	Steps
Automatic	Enter the mean of the sample absorbance values into a suitable data analysis software program to prepare the standard curve, see section, **Plotting the standard curve**. *The data analyte program will automatically determine the analyte concentration by comparing the absorbance of the sample to the standard curve.*
Manual	Locate the mean sample absorbance on the Y-axis of the standard curve and read from the X-axis, the analyte concentration that corresponds to the specific absorbance value.

Handling very concentrated samples

Dilute samples exceeding the measuring range with Incubation buffer (Bottle 5) and repeat the ELISA. This dilution factor must be considered when calculating the content of MIA.

Typical results

The standard curve must be determined individually for each experiment. An example is shown in Figure 1.

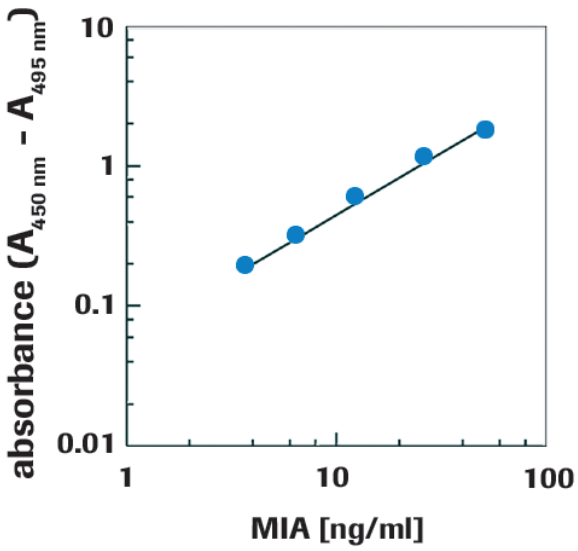

Fig. 1: Typical standard curve.

4. Troubleshooting

Observation	Possible cause	Recommendation
Unexpected color development.	Inadequate incubation time and temperature.	Ensure that incubation intervals are correct and that all reagents equilibrate to +15 to +25°C prior to use.
	Poor quality water negatively influences the test.	Always use double-distilled water for reconstitution and preparing the working solutions. ⚠ ***Water must not be microbially contaminated.***
	Substrate or vial used to aliquot substrate contaminated with oxidative active substances.	Do not pipette directly from the substrate bottle.
		Check the vial for contamination.
	Inadequate concentration of conjugate in the Immunoreagent.	Adjust to the correct concentration of the detection antibody in the Immunoreagent.
Questionable readings obtained.	Nonsuitable filters in the Microplate reader have been used.	Check the filters in your Microplate reader for the correct wavelength.
Weak or no signal present.	Sodium azide, 2-mercaptoethanol, and DTT interfere with the peroxidase activity.	Only use samples and solutions without sodium azide, 2-mercaptoethanol, or DTT.
Drift	Unequal distribution of temperature in the wells.	Ensure that all reagents equilibrate to +15 to +25°C prior to the assay.
		Use the recommended incubation times and temperatures.
	Evaporation of fluids.	Check for adequate placement of the adhesive cover foils during the incubation steps.
Poor precision	Non-homogeneous sample after freezing.	Mix sample completely before pipetting.
	Turbidity, particles, or high lipid content of the sample.	Centrifuge sample to pellet particles.
		Mix sample well before pipetting.
	Carryover between samples and standards.	Change pipette tips between each pipetting step.
	Unequal volumes added to the wells.	Check pipette function, and recalibrate if necessary.
	Inadequate aspiration of fluids.	Aspirate completely; no fluid should remain in the wells after aspiration.
	Washing was incomplete.	Ensure that the automatic washer is working properly.
	Unequal mixing of reagents during incubation.	Use a plate shaker to ensure adequate mixing.

5. Additional Information on this Product

5.1. Test Principle

Test principle

1. MIA protein is simultaneously bound by the biotin-labeled capture antibody and the peroxidase-conjugated detection antibody.
   – This complex binds via the biotin-labeled antibody to the streptavidin-coated surface of the microplate (one-step system).

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2. Following the washing step, the peroxidase (POD) bound in the complex is developed by ABTS as substrate and determined photometrically.
   – The developed color is proportional to the concentration of MIA protein.

How this product works

A number of research studies indicate that MIA, a 11 kDa soluble protein, is secreted by malignant melanoma cells. Literature supports evidence of the following:

- MIA expression pattern is highly cell type-restricted. In non-neoplastic tissues, MIA mRNA is mainly limited to developing and mature cartilage.
- When comparing the expression in normal skin, benign human skin melanocytes, benign melanocytic nevi, and malignant melanomas, MIA mRNA levels appear to parallel progressive malignancy of melanocytic tumors.
- High MIA mRNA levels were detected in almost 100% of malignant melanoma samples.
- MIA serum levels seem to be significantly and specifically increased in individuals with malignant melanomas.

Obviously, besides malignant melanoma cells, chondrocytes could also be a potential source for MIA. Therefore, degenerative diseases of the cartilage could also be a cause for elevated MIA levels. The MIA ELISA is intended as a tool to increase the scientific knowledge about these relationships.

Background information

Genetic properties

The human gene for MIA (initially identified as a melanoma inhibitory activity) is a single-copy gene mapped to human chromosome 19q13.32-13.33. The gene consists of four small exons interrupted by three introns encompassed within a small region of approximately 2 kb. More recently, MIA mRNA was identified independently by a differential display approach and has been referred to as CD-RAP.

Molecular properties

MIA is translated as a 131-amino acid precursor and processed into a mature 107-amino acid protein after cleavage of a putative secretion signal. The native protein seems to be secreted as a small globular protein stabilized by two intramolecular disulfide bonds. All four cysteine residues involved are conserved between the human and murine sequence, which exhibit 88% amino acid identity. No sequence homology to any known protein has been detected so far.

Biological function

MIA has been identified and subsequently purified as a melanoma-inhibiting activity secreted by HTZ-19 cells (malignant melanoma cell line established from a central nervous system melanoma metastasis). The biological function on molecular level is still barely understood. However, it is speculated that the function of this growth-regulatory protein is to slow tumor progression *in vivo* by an autocrine mechanism.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Trademarks

ABTS is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany