



## M22 (MUC1)



**Enzyme-immunoassay for the quantitative  
determination of MUC1 glycoprotein  
in serum or plasma**

REF

GD7295 00

IVD

### INDICATION

MUC1 is a heterogeneous glycoprotein with a molecular mass ca. 300-450 kD. Elevation of serum MUC1 is associated with mammary carcinomas. Quantitative determination of MUC1 in serum and plasma is helpful in monitoring of patients with such tumors to estimate the course of the disease, effectiveness of its treatment and to reveal recurrence or metastases. However, MUC1 values obtained should always be interpreted in context of results obtained by other diagnostic and clinical procedures.

MUC1 levels may also rise other tumors (lung tumors, prostate cancer, ovarian carcinomas, gastro-intestinal tumors). Elevation of MUC1 level can be also found in endometriosis, hepatitis, liver cirrhosis and lung fibrosis, pregnancy and lactation.

### PRINCIPLE OF THE ASSAY

The present test is a sandwich enzyme immunoassay on solid phase (ELISA). Patient specimen is placed into the microwells of a microplate coated with anti-MUC1 antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface, while unbound material is removed by washing. During a second incubation, antibodies directed towards MUC1 antigen and labelled with peroxidase enzyme (HRP), are added into the microwells. After incubation unbound labelled antibodies are removed by washing, while the remaining enzymatic activity bound to the microwell surface is detected by addition of chromogen-substrate solution. Developed colour, revealed at 450 nm, is directly related to the quantity of the analyte present in the specimen. The optical density values measured for the standards are used to generate a calibration curve from which the analyte concentration in the sample can be ascertained.

### KIT CONTENT

#### 1. Reagent A – Microplate

12x8 strips.

8 wells breakable strips, coated with antibodies anti-MUC1. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

#### 2. Reagent B – Enzymatic Tracer

1 vial of 11 ml.

Ready to use liquid reagent containing monoclonal antibodies anti-MUC1 labelled with Horseradish peroxidase (HRP) diluted in Phosphate buffer with 0.1% Phenol as preservative.

#### 3. Reagent C – Washing Solution 21x

1 vial of 22 ml.

Concentrated solution to be diluted 1:21 with distilled water. It contains detergent (Tween-20) and preservative (ProClin 300).

#### 4. Reagent D/E – Chromogen/Substrate

1 vial of 11 ml.

Ready to use solution containing Tetramethylbenzidine (TMB) with activators and stabilizers.

**Avoid light exposure.**

#### 5. Reagent F – Stop Solution

1 vial of 11 ml.

Ready to use solution containing a solution of Sulphuric acid (5.0% vol/vol).

**Avoid any skin contact.**

#### 6. M22 Standards

6 vials of 0.8 ml each.

Ready to use liquids containing, in Phosphate buffer with 0.1% Phenol as preservative, human MUC1 at the following concentrations:

**S<sub>0</sub>**: 0 U/ml, **S<sub>1</sub>**: 5 U/ml, **S<sub>2</sub>**: 10 U/ml, **S<sub>3</sub>**: 20 U/ml,  
**S<sub>4</sub>**: 50 U/ml, **S<sub>5</sub>**: 100 U/ml.

#### 7. M22 Control

1 vial of 0.8 ml.

Ready to use liquid reagent containing diluted human serum with high content of MUC1 and 0.1% Phenol as preservative. Concentration range is stated on the label.

#### 8. Reagent G – Blue Buffer

1 vial containing 11 ml.

Blue ready to use liquid containing Phosphate buffer and 0.1% Phenol as preservative.

#### 9. Cardboard sealers

2 cardboard sealers to be used to cover the plate during the incubations.

#### 10. Package insert: instruction for use GD7295 00 it/ing.

## MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

## STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

## AUXILIARY MATERIALS

- Semi automatic pipettes of 50-1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm (620- 630 nm).
- Microplate shaker (600-800 RPM)
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

## SAMPLES

Serum or plasma (in ACD or eparin). Specimens can be stored up to 48 hours at 2-8 °C before testing; for a long storage, the specimens should be frozen at -20 °C. Repeated freezing/thawing should be avoided. Turbid, hemolytic, lipemic, or contaminated microbiologically samples should be avoided.

## REAGENTS PREPARATION

- **WASHING SOLUTION:** dilute 1:21 with distilled or ELISA grade water (e.g.: 1 ml of Reagent C + 20 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for 5 days at room temperature or 30 days at +2-8 °C. It is recommended to store diluted washing solution at room temperature for immediate use.

## WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

## ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards, control and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards, control and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blanking" of the instrument is to be carried out in the Standard 0 U/ml.

**ASSAY SCHEME**

- Put the desired number of microstrips into the frame.
- If suggested analyte concentration in the sample exceeds 100 U/ml, dilute the sample using Reagent G (Blue Buffer).
- Follow the scheme:

	Microwells coated with antibody anti-MUC1		
	REAGENTS	Standard/Control	Sample
First incubation	Reagent G (Blue Buffer)	50 µl	50 µl
	Standard/Control	50 µl	-
	Sample	-	50 µl
	- Cover the strips with cardboard sealer - Incubate <b>30 minutes</b> at <b>18-25 °C, continuous shaking (600-800 rpm)</b>		
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 3 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid		
Second incubation	Reagent B (Tracer)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate <b>30 minutes</b> at <b>18-25 °C, continuous shaking (600-800 rpm)</b>		
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid		
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate <b>10-20 minutes</b> at <b>18-25 °C, avoiding light exposure</b>		
	Reagent F (Stop Solution)	100 µl	100 µl
	Read the absorbance of each well against Blank (Standard 0) at 450 (and 620-630 nm)		

**CALCULATION OF RESULTS**

- Calculate the mean value of the OD 450 nm obtained for each duplicate.
- Subtract blank value (Standard 0) to the mean OD 450 nm values of standards, control(s) and sample.
- Draw a standard curve by plotting the absorbances of the standards with the corresponding concentrations. A point-by-point method for data reduction is recommended. Alternatively, the calculation system of the microplate reader software can be used.
- Calculate the concentrations of control(s) and samples from the obtained standard curve.

**QUALITY CONTROL**

M22 Control concentration should fit into the established range stated on the labels.

It is important to always include, within the test procedure, commercial controls with known M22 concentrations for validating the accuracy and the precision of the test.

The test results are valid only if all controls are within the specified ranges.

**EXPECTED VALUES**

Based on data obtained by AMS, the following normal ranges are recommended (see below). However, it is recommended that each laboratory establish its own reference range.

Subjects	M22, U/ml	
	Lower limit	Upper limit
Men		30
Women		30
Pregnancy 1 <sup>st</sup> trimester		65
2 <sup>nd</sup> trimester	2.0	80
3 <sup>rd</sup> trimester	2.0	235
Lactation		150

The patients that have received murine monoclonal antibodies for radioimaging or immunotherapy may develop high titers of anti-mouse antibodies (HAMA). The presence of these antibodies gives false results in the present assay. Sera from HAMA positive patients should be treated by depleting adsorbents before assaying.

**Note:** Therapeutic consequences should not be based the results obtained by this method alone; all available clinical and laboratory findings should be used by physicians to elaborate therapeutically measures.

## ANALYTICAL PERFORMANCES

### Sensitivity

The lowest detectable concentration of MUC1 is 1.5 U/ml.

### Precision

Intra-assay: %CV ≤ 6.8

Inter-assay: %CV ≤ 7.6

### Linearity

Linearity was checked by assaying serial dilutions of 5 samples with different MUC1 (M22) concentrations. Linearity percentages obtained ranged within 90 to 110%.

### Recovery

Recovery was checked by assaying 5 samples spiked with known MUC1 (M22) concentrations. The recovery percentages obtained ranged within 90 to 110%.

### Analytical Specificity / Cross-reactivity

Analyte:	Cross-reactivity, % wt/wt
CA125	< 0.1
CEA	< 0.1
CA19.9	< 0.1

## PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium aide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended <sup>(6)</sup>.

### Waste Management

Please refer to local legal requirements.

## REFERENCES

1. McGuire WL, Tandon AK, Allred D, Chamnes GC, Clark GM: How to use prognostic factors in axillary node negative breast cancer patients. J Natl Cancer Inst 1990; 82:1006-7.
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6. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.