

# IMTEC-ANA SCREEN

## ANA Screen

### ELISA for the Quantitative Determination of Antinuclear Antibodies (Ig(GAM))

#### Package Size

<b>REF</b>	ITC60001	96 Tests	Complete Testkit
<b>IVD</b>			

Please read the instructions carefully before testing.

#### Procedural precautions:

Do not use the reagents beyond the date of expiry.

**DIL DB07, WASH|20x| WB03, SUB TMB ELISA and STOP STOP ELISA may be interchanged between lots and test kits that share the same reagent designation.**

**All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits**

**Store reagents at 2...8°C.**

#### Intended Use

IMTEC-ANA Screen is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative determination of autoantibodies against nuclear antigens in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of connective tissue diseases.

Antinuclear antibodies (ANA) are autoantibodies of different specificity directed against antigens of the cell nucleus. In general ANA can be divided into antibodies aiming at extractable nuclear antigens (ENA), non-extractable nuclear antigens and cytoplasmatically located antigens.

The detection of ANA is important for diagnosis of collagenosis especially of systemic lupus erythematosus (SLE) and the "mixed connective tissue disease (MCTD)" that is strongly associated with SLE, and other rheumatic diseases also. In case of SLE and MCTD ANA are detectable at almost 100% of all cases.

#### Principle

The test is based on the covalent immobilisation of HeLa cell nuclei, carefully isolated by density gradient centrifugation, to the solid phase of microtiter strips (patented) and subsequent binding of ANA from patient serum. The bound antibodies are detected with a peroxidase-labelled secondary antibody that is directed against human IgG, IgM and IgA. After addition of substrate solution, a colour appears which intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

#### Reagents and Contents

<b>MTP</b>	12	<b>Microtiter Strips</b> (in 1 strip holder) 8-well snap-off strips, ready for use coated with HeLa cell nuclei
<b>CAL</b>	1 – 5 5 x 1.5 ml	<b>Calibrators IgGAM</b> (white cap), human serum, inked according to concentration, ready for use ANA level: 31.25 U/ml (1), 62.5 U/ml (2), 125 U/ml (3), 250 U/ml (4), 500 U/ml (5)
<b>NC</b>	1.5 ml	<b>Negative Control Serum</b> (green cap), human, ready for use
<b>PC</b>	1.5 ml	<b>Positive Control Serum</b> (red cap), human, ready for use Concentrations are stated on the labels.
<b>WASH 20x  WB03</b>	50 ml	<b>Washing Buffer</b> (black cap) Concentrate (20x) for 1 l TRIS buffer pH 6.9 ± 0.2
<b>DIL DB07</b>	100 ml	<b>Dilution Buffer</b> (blue cap) ready for use Phosphate buffer pH 7.2 ± 0.2
<b>CON</b>	15 ml	<b>Conjugate Solution</b> (white cap) anti-human-IgGAM HRP conjugate, ready for use
<b>SUB TMB ELISA</b>	15 ml	<b>TMB solution</b> (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidine Hydrogen peroxide pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l

<b>STOP</b>	15 ml	<b>Stop Solution</b> (red cap) Sulphuric acid, ready for use 0.5 mol/l
<b>STOP ELISA</b>	1	<b>Adhesive Strip</b>

#### Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

**STOP**, **SUB** can irritate eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

#### Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

#### Reagent Preparation

**Allow the testkit and all its components to reach room temperature before use!** Used bottles should be closed carefully and stored at 2...8°C. Store **SUB** protected from light.

Do not use polystyrene vessels for handling of **CON**.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

#### Washing Buffer Solution **WASH**

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part **WASH|20x|** with 19 parts distilled water. **WASH** is stable for 6 weeks stored at 2...8°C.

#### Specimen

Patient sera

Use samples freshly collected or freeze samples at -20°C. **Freeze and thaw once only.** Do not use serum samples inactivated by heat treatment at 56°C.

Allow the samples to reach room temperature (30 min.).

Dilute sera 1:101 with **DIL** (add 10 µl serum to 1 ml **DIL**).

#### Procedure

- **Pipette 100 µl** diluted sample, **CAL**, **PC** and **NC** into **MTP**, for blank use **DIL** instead of sample dilution, seal **MTP** with adhesive strip.
- Incubate for **1 hour** at RT.
- Discard the solution from **MTP**. Wash **MTP** 3 times using 300 µl **WASH** per well.
- Discard **WASH** and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl CON** and seal **MTP** with adhesive strip.
- Incubate for **30 min.** at RT.
- Discard the solution from **MTP**. Wash **MTP** 3 times using 300 µl **WASH** per well.
- Discard **WASH** and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl SUB** and incubate for **10 min.** At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 min..
- **Add 100 µl STOP** per well.
- Read absorbance values at 450 nm within the next 10 min. after stopping. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended.

#### Automation

The IMTEC-ANA Screen ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated prior to diagnostic use.

#### Validation of the Test

The test results are valid provided the following criteria are met for the obtained results:

- **PC** is within the indicated range (see label).
- **NC** is lower than the cut-off-value of the test.
- **CAL|5** does not fall below an absorbance value of 0.6.
- The absorbances of **CAL|1-5** keep raising.

In order to improve accuracy of the test results we recommend to run **CAL|1-5**, **PC**, **NC** and patient samples in duplicate.

### Interpretation of Results

Plot measured absorbances against U/ml of [CAL]1-5 in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of the ANA in the patient samples can be determined.

Results below 40 U/ml (cut-off value) are considered negative. Results between 40–55 U/ml are equivocal and above 55 U/ml positive.

### Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

Elevated antinuclear antibodies may occur in individuals with no evidence of clinical disease.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

The performance characteristics for this assay have not been established for plasma samples.

### Performance characteristics

Typical performance data can be found in the Verification Report, accessible via:

[www.human.de/data/gb/vr/el-60001.pdf](http://www.human.de/data/gb/vr/el-60001.pdf) or

[www.human-de.com/data/gb/vr/el-60001.pdf](http://www.human-de.com/data/gb/vr/el-60001.pdf)

### References

1. Fritzler M.J., Mol. Biol. Rep. **23**, 133 (1996)
2. Muro Y., Autoimmunity **38**, 3 (2005)
3. Tonutti E. *et al.*, Autoimmunity **37**, 171 (2004)

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**Human**