ASPERGILLUS FUMIGATUS

Enzyme immunoassay for the diagnosis of aspergillosis by *Aspergillus fumigatus*

96 assays on individual wells for in vitro use

Instructions for use for article N° **6100**
EC reg. N°: CH-201301-0006

CE

Intended use:

Serological diagnosis (IgG) of aspergillosis.
Follow up of patients at risk of aspergillosis infections.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with the following mix:

- Soluble somatic and metabolic *Aspergillus fumigatus* antigens
- Recombinants antigens: dipeptidylpeptidase type V (chymotrypsin) and ribonuclease (mitogillin) from *Aspergillus fumigatus*

The presence of mycosic specific serum antibodies is detected with a Protein A - alkaline phosphatase conjugate. Sensitized wells are provided as breakable strips for the economical assay of small series of samples.

Material contained in the kit (96 assays):

WELL	6100-01	Breakable ELISA strips sensitized with ***Aspergillus fumigatus*** antigens	96	wells
DILB	6100-02	Dilution buffer (10 x) concentrate	50	ml
WASH	6100-03	Washing solution (10 x) concentrate	50	ml
ENZB	6100-04	Enzyme buffer	50	ml
STOP	6100-05	Stopping solution (K_3PO_4)	25	ml
CONTROL -	6100-06	Negative control serum	200	µl
CONTROL -/+	6100-07	Weak positive serum (cut off)	200	µl
CONTROL +	6100-08	Positive control serum	200	µl
CONJ	6100-09	Protein A - alkaline phosphatase conjugate	300	µl
SUBS	6100-10	Phosphatase substrate	20	tablets
		Multipipette reservoir, 25 ml	1	piece
		Frame for ELISA 8-well holder	1	piece

Shelf life and storage:

Store kit at 2° to 8° C (transport at ambient temperature). The expiry date and the lot number of the kit are printed on the side of the box.

Equipment needed but not provided with the kit:

Pipettes (ml and µl). Flasks. Tubes for the dilution of sera. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37° C. ELISA reader set at 405 nm.

Preparation of reagents before use:

ELISA wells: open side of aluminum bag 6100-01 and remove number of wells needed. Place sensitized wells in 8-well holder(s). If needed, complete the empty positions in the holder with used wells. Insert holder(s) in the frame in the correct orientation. Reseal open package with desiccant pad.

Dilution buffer: dilute dilution buffer (10 x) concentrate 6100-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 6100-03, 1/10 in distilled water. You may also use your own washing solution. Avoid buffers containing phosphate which could inhibit the enzymatic activity of the alkaline phosphatase.

Negative, weak positive(cut off) and positive **control sera:** dilute 10 µl control sera 6100-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20).

Sera to be tested: dilute 10 µl serum in 2.0 ml dilution buffer solution (final dilution 1/201).

Protein A - alkaline phosphatase **conjugate:** dilute conjugate 6100-09 in dilution buffer solution (final dilution 1/51).

Substrate solution: prewarm enzyme buffer 6100-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate 6100-10 in undiluted buffer 6100-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

Stopping solution: use reagent 6100-05 undiluted.

Warnings and precautions: Solutions 6100-02, 6100-03, 6100-04 and 6100-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% sodium azide (N_aN_3). Solution 6100-02 contain 0.02% merthiolate. These substances are toxic. The stopping solution 6100-05 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera (6100-06 to –08) are from rabbits.

Volumes to be prepared:

			Total number of wells to be used			
			3-4	**5-6**	**7-8**	**9-10**
Dilution buffer (10 x)	6100-02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	6100-03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	6100-09 + dilution buffer	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	6100-06 to -08 + dilution buffer	µl + µl	10 + 190	10 + 190	10 + 190	10 + 190
Sera to be tested	Serum + dilution buffer	µl + µl	10 + 2000	10 + 2000	10 + 2000	10 + 2000
Substrate solution	6100-10 + 6100-04	tabl. + ml	1 + 2.5	1 + 2.5	1 + 2.5	1 + 2.5

Procedure:

Step 1: Blocking:

Fill completely wells with dilution buffer solution.

Incubate for 5 to 15 minutes at ambient temperature (blocking).

Remove dilution buffer by aspiration or by shaking the strips over the sink.

Step 2: Incubation with serum samples:

Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank).

Fill the subsequent three wells with 100 µl diluted negative, weak positive (cut off) and positive control sera respectively (100 µl each).

Fill remaining wells with the diluted sera to be tested (100 µl each).

Cover wells with adhesive tape and incubate for 30 minutes at 37° C.

Remove sera and wash 4 x with washing solution.

Step 3: Incubation with conjugate:

Distribute 100 µl diluted protein A - alkaline phosphatase conjugate in each well.

Cover wells with adhesive tape and incubate for 30 minutes at 37° C.

Remove conjugate and wash 4 x with washing solution.

Step 4: Incubation with substrate:

Distribute 100 µl substrate solution per well.

Cover wells with adhesive tape and incubate for 30 minutes at 37° C.

Stop the reaction by the addition of 100 µl stopping solution to each well.

Step 5: Measurement of absorbances:

Wipe bottom of wells, eliminate bubbles and measure absorbances at 405 nm.

Interpretation:

Subtract value of the no-serum blank from all measured values. The test is valid if the following criteria are met: absorbance (A) of positive control > 1.200, A of negative control < 10% of A of positive control, A of blank against air < 0.350.

The antibody concentration of the weak positive serum 6100-07 has been set to discriminate optimally between sera of clinically documented cases of aspergillosis in immunocompetent patients and healthy human sera.
The cut off index of a sample is defined, after subtraction of the no-serum blank, as:

$$\text{Index} = \frac{\text{Absorbance sample}}{\text{Absorbance cut off serum}}$$

The result is **negative** when the index of the analyzed sample is lower than **1.0**. In this case, the IgG antibody concentration against ***Aspergillus fumigatus*** antigens is clinically non-significant.
A grey area correspond to an index comprised between **0.8** and **1.0**. In this case, the sample is considered as borderline, it is recommended to repeat the test with the same sample or with a new serum of the same patient, taken after 2-4 weeks.
The result is **positive** when the index of the analyzed sample is higher than **1.0**. In this case, the IgG antibody concentration against ***Aspergillus fumigatus*** antigens is considered as clinically significant. This result leads to an aspergillosis or an aspergillosis sensitisation.
In each case, results must be correlated to radioclinicals observations on the patient.
In case of immunosupressed patients, it is recommended to complete the test with the detection of *A. fumigatus* antigens in serum.

This quantitative and reproducible test allow the follow up of the serological evolution for a given patient.

Sensitivity and specificity of the assay:

A **sensitivity** of **97%** was found with 32 sera from 19 patients suffering from various aspergillosis (11 chronic necrotizing aspergillosis, 12 aspergilloma, 2 aspergillus sinusitis and 7 allergic bronchopulmonary aspergillosis). A sensitivity of 22% was found with 9 sera from 5 patients suffering from invasive aspergillosis.
A **specificity** of **98%** was found with 131 sera from 67 patients suffering from non aspergillus respiratory diseases (candidosis, tuberculosis, pneumocystosis, cryptococcosis, viral or bacterial pneumonia.)

Repeatability were assessed by testing 2 human serum samples in 24 wells on 1 assay.
Reproducibility were assessed by testing the 2 human serum samples on 10 differents assays.

	Repeatability		**Reproducibility**	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Average (OD value)	0.352	1.767	0.410	1.985
Standard deviation (OD value)	0.027	0.069	0.038	0.096
Variation coefficient (%)	7.6	3.9	9.3	4.8

References:

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Sarfati, S., Monod, M., Recco, P., Sulahian, A., Pinel, C., Candolfi, E., Fontaine, T., Debeaupuis, J.P., Tabouret, M., Latgé, J.P. (2006) Recombinant antigens as diagnostic markers for aspergillosis. Diag. Microbiol. Inf. Disease **55**, 279-291.

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