GAD II IgG

Order Code: GADII-24

Antigen Chip

BlueDiver Protocol: 02

BlueDiver Combi reference: **046**

1 INTENDED USE

D-tek's "Combi" system allows the custom assembly of different antigen chips into a same test strip. BlueDiver Combi GAD II IgG is part of an immunodot test kit. It is composed of a plastic *dispenser* containing 24 breakable antigen chips. In the present case, they are intended for the detection of IgG autoantibodies against GAD II in human sera.

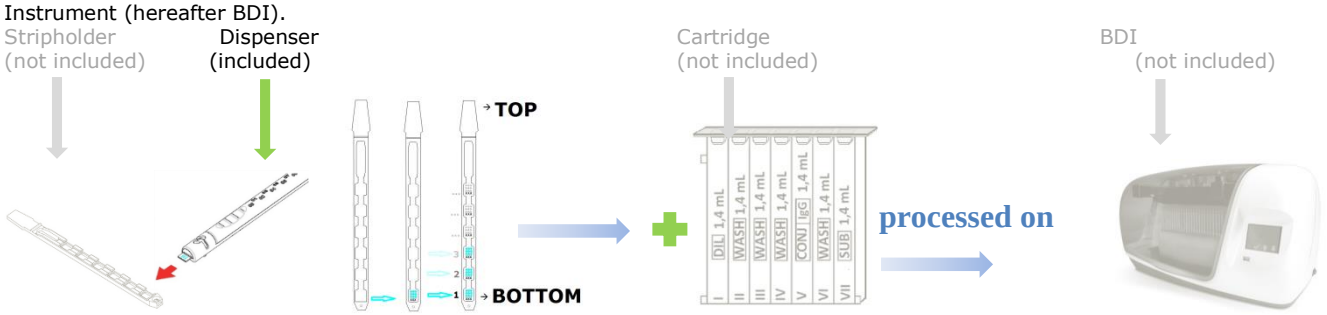
Please note that D-tek's GADII-24 is for the detection of **Stiffman Syndrome only** and should not be used for the diagnosis of diabetes mellitus type 1 (T1D).

More information on the source/type of antigens is available via your distributor or via our website www.d-tek.be.

2 PRINCIPLE OF THE TEST

A BlueDiver Combi test requires: antigen chip(s), stripholder(s) and reagent cartridge(s). Stripholders and reagent cartridges must be ordered apart from antigen chips.

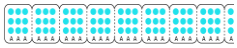
The antigen chips are contained in a dispenser. Each dispenser contains 24 breakable antigen chips. The user chooses the antigen chip(s) he/she wants to test and assembles them on an empty stripholder (one stripholder per patient to test and up to 6 antigen chips per stripholder – ALWAYS LOAD FROM BOTTOM TO TOP!!). The mounted stripholders are used in combination with cartridges containing the ready-to-use reagents for the test (diluent buffer, wash buffer, conjugate and substrate). The test has to be performed on BlueDiver Instrument (hereafter BDI).



The test is based on the principle of an Enzyme Immunoassay. During the automated test procedure, the BDI sequentially incubates the strips in the wells of ready-to-use reagent cartridges. Briefly: the strips are first incubated with diluted patients' sera. Human antibodies, if present, bind to the corresponding specific antigen(s) dotted on the membrane. Unbound or excess antibodies are removed by washing. Upon further incubation into AP-conjugated goat antibodies against human IgG, the enzyme conjugate binds to the antigen-antibody complexes. After removal of excess conjugate by washing, the strips are finally incubated into a substrate solution. Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.

3 CONTENTS

Prior to any use of the kit, please check that all the items listed are present.
If one of the items is missing or damaged, please do not use the kit and contact your distributor.

1 dispenser unit  containing Σ 24 x  (breakable antigen chip) 

4 MATERIAL REQUIRED BUT NOT PROVIDED

Reagent cartridge(s), empty stripholder(s), BDI (minimum software version 85), Dr DOT Software (minimum version 3.7), micropipettes, laboratory gloves, mechanical guide for insertion of antigen chip in stripholder (optional).

5 STORAGE

The dispenser must be stored at a temperature between +2°C to +8°C. Do not freeze.

After initial opening of the dispenser, please store at 2-8°C protected from (sun)light preferably inside the aluminium pouch and the original box.

When stored properly, the test kit component is stable until the indicated expiry date.

6 SAFETY PRECAUTIONS

The reagent inside this dispenser is for in vitro diagnostic and professional use only and should be processed by trained technical staff only.

The dispenser contains potentially hazardous components, thus contact with skin, eyes or mucosae has to be avoided.

Patient samples shall be handled with care as being a potential infection hazard.

Waste disposal: Patient samples, incubated test strips and incubated cartridges should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

D-tek s.a. and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated.

In any case, GLP should be applied with all general and individual regulations to the use of this kit.

7 SAMPLE COLLECTION, HANDLING AND STORAGE

Serum or plasma can be used in the test. Blood samples can be collected in dry tubes or in tubes containing EDTA, heparin or citrate. After separation serum or plasma samples can generally be stored at 2-8°C for up to three days. Long term storage requires freezing at -20°C. Avoid repeated freezing/thawing cycles. After freezing always agitate samples before use to ensure homogeneity.

8 ASSAY PROCEDURE

BASIC INFORMATION, HANDLING AND TIPS:

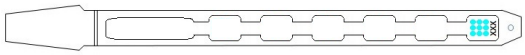
TEST PROCESS principle:

Only kits having the same protocol number can be processed simultaneously on the BDI (they can be of the ranges BlueDiver Quantrix, BlueDiver Dot and BlueDiver Combi). Please refer to the protocol reference underneath the Kit Name and reference on page 1.

After manual loading of the antigen chips into the stripholders, the prepared stripholders and the reagent cartridges can be loaded into the BDI. The incubation and washing steps of the procedure are automatically processed by the BDI. The continuous up and down movement ensures an efficient circulation of fluids over the strips in the wells of ready-to-use reagent cartridges. The whole test procedure is run at room temperature.

STRIPHOLDER description:

The reactive (front) side of the stripholder shows the coated side of the individual antigen chips that have been mounted (they show faint blue dots). This coloration ensures that all antigens have been correctly spotted onto the membrane. The coloration disappears during the processing of the test. This front side also displays underneath the 3 lines of dots the BlueDiver Combi reference (Please refer to the BlueDiver Combi reference underneath the Kit Name and reference on page 1). ALWAYS load the stripholders from BOTTOM to TOP!!



The non-reactive (back) side of the stripholder displays 2-dimensional square barcodes for each antigen chip. This guarantees the traceability of the antigen chips used after removal from the BDI at the end of the test.

The mounted strips must be manually inserted into the dedicated clamp before starting the automated process (see Test Preparation hereafter, pt 8.1.4). During this operation, avoid touching the membrane zone of the strips with fingers. Always wear laboratory gloves and use the plastic parts (strip support) for handling or manipulation.

REAGENT CARTRIDGES description:

The reagent cartridges are composed of 7 different wells filled with ready-to-use reagents. The cartridges are sealed, and the reagent wells are hermetically separated. The sealing has to be removed before starting the test. Once opened, manipulate the cartridges with care in order to avoid reagent spilling and contamination from well to well. The rear (back) side of the cartridges is labelled with both alphanumeric and bar-coded information for identification of the cartridge type and lot number by the BDI.

The cartridges must be manually loaded onto the dedicated cartridge holder before starting the automated process (see Test Preparation hereafter, pt 8.1.10). The front and rear (back) sides of the cartridges have, respectively, a bottom triangular and two (bottom + top) square plastic edges for secure position and orientation into the holder.

8.1 Test preparation

- Allow all kit components to equilibrate at room temperature (+18°C to +25°C) before use.
- A working list (either edited from Dr DOT software, or external) should always be prepared for easy loading and correct association of antigen chips, strips, cartridges and patient samples.
- Make sure that the cartridge holder is fixed in its emplacement in the BDI.
- Make sure that the BDI is plugged in.
- Make sure that all the tests that you wish to run do have the same protocol number.

The following steps sequence summarizes the loading and preparation of the BDI, test strips, reagent cartridges and patient samples before starting the test. For detailed information or in case of any problem met at one of the following steps, please refer to the Manual of Use of the BDI.

1. Switch ON the BDI and wait a few seconds until the date and time are displayed on the touch screen.
2. Confirm the correct Date and Time by pressing ✓ on the touch screen (in case of first use or for reset, refer to the manual of use of the BDI) → "**Initialize?**" is displayed on the screen.
3. Confirm Initialization by pressing ✓ on the touch screen → the horizontal arm of the instrument automatically moves forward to a central (stand-by) position → "**Load strips (24")** is displayed on the screen
4. (Do not set nor confirm the number of strips at this step). Remove the clamp from its emplacement on the arm by gently pulling it upwards and load the strips to be tested: handle the clamp with numbered side facing up (open position) and insert the strips, also with numbered (reactive) side facing up, by slipping the upper plastic part (tongue) into the dedicated holes of the clamp. Apply a gentle pressure to ensure that the plastic tongue has reached the bottom end of the hole.
   Notes:
   - Always start loading into position 1 of the clamp (left side) and do not leave empty spaces between the strips!
   - After complete loading, check visually the vertical, horizontal and lateral alignment of the strips. Any obvious misalignment should be corrected by unloading the strip(s) from the clamp and loading them again.
   - Be careful: any plastic bits remaining after breaking apart the individual strip holders may hinder the processing on the instrument and/or the reading with the BlueScan scanner; please remove them with scissors.
5. Replace the clamp in its emplacement on the arm by gently pushing it downwards.
6. Set the number of loaded strips using the up and down arrows on the touch screen.
7. Confirm the number of loaded strips by pressing ✓on the touch screen → the horizontal arm automatically moves backwards to stand over the alignment holes of the cartridge holder → "**Check alignment**" is displayed on the screen.
8. Use the "JOG" function on the screen to check the correct alignment of the strips: maintain a gentle pressure on the down arrow on the touch screen until the bottom of the strips enters into the alignment holes of the cartridge holder. If correctly aligned, the strips will not touch the outlines of the holes.
   Note: in case of misalignment (contact of the strips with the cartridge holder), please refer to the Manual of Use of the BDI).
9. Confirm the correct alignment of the strips by pressing ✓on the touch screen → the BDI lowers the strips completely into the alignment holes and process to the reading. Please note that BlueDiver Combi strips cannot be read by the BDI so the LED will not flash. After the process of reading, "**Load reagent**" is displayed on the touch screen.
10. Unseal the reagent cartridges and insert them under their respective strips in the dedicated notches of the cartridge holder.

11. Confirm complete loading by pressing ✔on the touch screen → the BDI reads the cartridges barcodes. After complete barcode reading, the number of strips is displayed on the screen. The BDI identifies BlueDiver Combi strips position based on the specific cartridge barcode therefore the LED at positions corresponding to BlueDiver Combi strips will light on.
    Note: in case of failure to read one or more cartridge(s) barcode(s), please refer to the Manual of Use of the BDI.
12. Confirm the number of strips by pressing ✔on the touch screen → the protocol number identified on the barcodes is displayed on the screen (**Protocol ID 02.)**.
13. Confirm the protocol number by pressing ✔on the touch screen → "**Please close cover.**" is displayed on the screen.
14. Close the cover of the BDI and confirm closing by pressing ✔on the touch screen → the BDI proceeds to a first washing (pre-treatment) step by incubating the strips into the 2nd well of the cartridges (processing time: 1 minute) → At the end of the wetting step, "**Please open cover.**" is displayed on the screen.
15. Open the cover of the BDI and confirm opening by pressing ✔on the touch screen → the horizontal arm automatically moves forward to the front of the instrument and swings the strips to an oblique position → "**Dry strips"** is displayed on the screen.
16. Dry the strips by gently applying absorbent paper onto the basis of the bottom small cavity (sample loading hole).
17. Confirm drying by pressing ✔on the touch screen → "**Apply samples**" is displayed on the screen.
18. Apply samples by pipeting 10 µl of patient serum/plasma into the bottom sample loading holes of the strips.
    Note: If preferred, the 10µl of the serum can be directly pipetted into the Diluent Buffer ("Well I") of the cartridge. This operation can be done at any time from opening of the cartridges (see Point 8.1.10).
19. Confirm samples' loading by pressing ✔on the touch screen → "**Please close cover**" is displayed on the screen.
20. Close the cover of the BDI and confirm closing by pressing ✔on the touch screen → the BDI starts the test automatically by proceeding the following steps sequence (**Protocol 02**):

8.2 Test processing (Protocol 2)

Step	Description	Processing time
01.	The strips are incubated into the 1st well of the cartridge (*Diluent Buffer*). Upon contact with the liquid in the wells and agitation, the pre-loaded patients' samples (see 8.1.18) are released from the small cavity at the bottom of the strips and are diluted in the buffer.	30 min
02.	The clamp moves forwards and the strips are incubated into the 2nd well of the cartridge (*Wash Buffer*)	2 min
03.	The clamp moves forwards and the strips are incubated into the 3rd well of the cartridge (*Wash Buffer*)	2 min
04.	The clamp moves forwards and the strips are incubated into the 6th well of the cartridge (*Wash Buffer*)	2 min
05.	The clamp moves backwards and the strips are incubated into the 5th well of the cartridge (*Conjugate*)	10 min
06.	The clamp moves backwards and the strips are incubated into the 4th well of the cartridge (*Wash Buffer*)	2 min
07.	The clamp moves backwards and the strips are incubated into the 3rd well of the cartridge (*Wash Buffer*)	2 min
08.	The clamp moves backwards and the strips are incubated into the 2nd well of the cartridge (*Wash Buffer*)	2 min
09.	The clamp moves forwards and the strips are incubated into the 7th well of the cartridge (*Substrate*)	10 min
10.	The clamp moves backwards and the strips are incubated into the 6th well of the cartridge (*Wash Buffer*)	2 min

After completion of the process, the clamp moves to a central (stand-by) position in the BDI to allow easy manipulation of the clamp. The instrument beeps and "**Finished test**" is displayed on the screen.

Gently apply absorbent paper onto the basis of the strips to remove liquid from the bottom small cavity (sample loading hole) and allow the strips to dry for 30 minutes before interpretation of the results. The interpretation has to be done in the 24 hours following the test processing.
In case of use of the BlueScan for help of results interpretation, please leave the processed strips attached to the clamp.

TEST DATA REGISTRATION
The test protocol can be downloaded by pressing the USB stick symbol and following the indications on the screen (Insert USB → Writing USB → Remove USB). This step is not obligatory but is highly recommended for traceability and regulatory matters.

9 RESULTS INTERPRETATION

The evaluation of the results is performed via the Dr DOT Software and scanning system.
More information on Dr DOT is available via your distributor or via our website www.d-tek.be

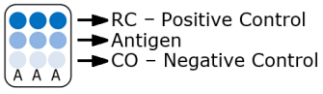
*NB: Dr DOT Software is an interpretation **supporting** software only. The final clinical interpretation has always to be validated by a professional clinician or physician.*

1. Remove the clamp from the BDI. Leave the processed strips attached to the clamp.
2. Insert the clamp, the reactive side of the strips facing down, into the dedicated emplacement in the cover of the BlueScan scanner.
3. Start scanning the strips using the Dr DOT software.
   For detailed information about the BlueScan and Dr DOT software please refer to the Manual of Use of your Dr DOT software.

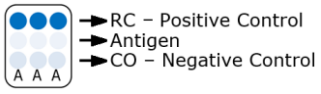
9.1 Antigen results

Each antigen chip contains integrated **Positive and Negative (cut-off) Controls** coated in triplicate; the Dr DOT software measures the mean colour intensity of each antigen triplicate and compares this intensity to the cut-off's intensity.

***POSITIVE RESULT*:**
A sample is considered positive for a specific antibody if the value of the corresponding antigen dots (spotted in triplicate on the antigen chip) is **higher than** the cut-off value (also spotted in triplicate on the antigen chip).
In its principal results sheet, the Dr DOT software highlights the antigens for which the result is positive and indicates the calculated numeric value into brackets.



***NEGATIVE RESULT*:**
A sample is considered negative for a specific antibody if the value of the corresponding antigen dots (spotted in triplicate on the antigen chip) is **lower than or equal to** the cut-off value (also spotted in triplicate on the antigen chip).



Dr DOT Arbitrary Unit (AU)	Interpretation
< 5	Negative
5 – 10	Equivocal (*)
>10	Positive

** Low titers of auto-antibodies may occur in healthy patients. For this reason low positive results (between 5 to 10 AU), although valid, should be considered equivocal. Retesting of the patient, preferably by using a new sample, is therefore recommended. If the result is still equivocal on retesting, then other diagnostic tests and/or clinical information should be used to help determine the autoimmune status of the patient.*

10 TEST PERFORMANCES

10.1 Reproducibility

Reference control samples were tested for each antibody in statistically relevant repetitions in a same run or over several runs for the calculation of intra- and inter-assay variation, respectively. In every case the intensity of the dots was within the specified range and standard deviations were less than 15 %.
Detailed analytical data are available upon request.

10.2 Sensitivity and Specificity

Characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies) were assayed following the test instructions. Sensitivity and Specificity were calculated from the results generated by the Dr DOT software.

GAD II

	+	-
+	true positive 54	false positive 0
-	false negative 0	true negative 200

Sensitivity	100%
Specificity	100%

Please note that D-tek's GADII-24 is for the detection of **Stiffman Syndrome only** and should not be used for the diagnosis of diabetes mellitus type 1 (T1D).

11 TEST LIMITATIONS

1. A clinical diagnosis should not be made on the basis of a single in vitro diagnostic method only.
2. A complete clinical investigation, as well as other laboratory test results, should be considered to state a diagnosis, since no technique used alone can rule out the possibility of false-positive or false-negative results. In this respect, more particularly an indirect Immunofluorescence test, when applicable, should be performed in parallel with the determination of autoantibodies by BlueDiver Dot, as Immunofluorescence is often considered as a gold reference screening technique in autoimmunity.
3. D-tek s.a. and its authorised distributors shall not be liable for any damages resulting from a change or modification in the procedure indicated, from misuse of the kit and/or from use of an incomplete or damaged kit. The kit should be performed by trained technical staff only.
4. In any case, GLP should be applied with all general and individual regulations to the use of this kit.
5. D-tek's liability shall in any event be limited to the replacement of the kit.

Version C,03/2018
Last revised: 03/2018