

Scleroderma⁸ IgG

Order Code: SCL8DIV-24
BlueDiver Protocol: 02

1 INTENDED USE

BlueDiver Dot Scleroderma⁸ IgG is an Immunodot kit intended for the detection in human sera of IgG autoantibodies against Scl-70, CENP-A, CENP-B, PM-Scl 100, PM-Scl 75, Ku, RNA Polymerase III and U1-RNP antigens.

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

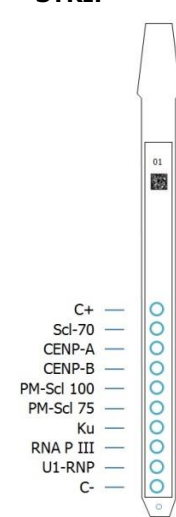
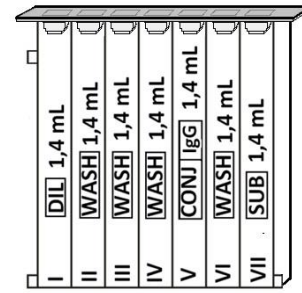
This kit is intended to be performed on the *BlueDiver Instrument* (hereafter BDI). The test is based on the principle of an Enzyme Immunoassay. The test strips are composed of a membrane fixed on a specific plastic support. During the automated test procedure, the BDI sequentially incubates the strips 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) 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 KIT CONTENTS

Abbreviations in alphabetic order:

AP = Alkaline Phosphatase; BCIP = Bromo-Chloro-Indolyl-Phosphate; KCl = Potassium Chloride; MgCl₂ = Magnesium Chloride;

MIT = MethylIsoThiazolone; NaCl = Sodium Chloride; NaN₃ = Sodium Azide; NBT = NitroBlue Tetrazolium; TBS = Tris Buffer Saline

		STRIP	CARTRIDGE
Dot strips	3 x 8 units on plastic supports breakable individually; sealed in aluminium pouch <u>10 dots on each:</u> 1 positive control (C+) 8 antigens 1 negative control (C-)		
Cartridge	24 units having each 7 compartments; sealed.		
Diluent buffer	I st position, 1 x 1,4 ml (yellow) <i>contains:</i> H ₂ O • TBS • NaCl • Tween • Preservative (MIT) • Dye • Antifoam emulsion		
Wash buffer	II nd , III rd , IV th and VI th position, 4 x 1,4 ml (colourless) <i>contains:</i> H ₂ O • TBS • NaCl • Tween • Preservative (MIT) • Antifoam emulsion		
Conjugate	V th position, 1 x 1,4 ml (red) <i>contains:</i> H ₂ O • TBS • NaCl • KCl • MgCl ₂ • AP-conjugated goat anti-human IgG • Stabilizer • Preservative (MIT) • Dye • Antifoam emulsion		
Substrate	VII th position, 1 x 1,4 ml (pale yellow solution) <i>contains:</i> H ₂ O • NaN ₃ (0.05 %) • MgCl ₂ • TBS • NBT • BCIP • Stabilizer • Antifoam emulsion		
Other material	Absorbent paper (for drying of tips pt 8.1.16), sealed Together with dot strips in aluminium pouch		
Documents	IFU, Certificate of Analysis, Antigen list		

4 MATERIAL REQUIRED BUT NOT PROVIDED

BDI, Micropipettes / Laboratory gloves

5 STORAGE AND STABILITY

The test kit must be stored at a temperature between +2°C to +8°C. Do not freeze. After initial opening of the kit, unused reagent cartridges must be stored at 2-8°C protected from (sun)light preferably inside the original kit box. Unused strips have to be placed back into the provided pouches, sealed and stored at 2-8°C preferably *inside* the original kit box. When stored properly, all test kit components are stable until the indicated expiry date.

6 SAFETY PRECAUTIONS

All reagents are for in vitro diagnostic and professional use only. The test kit should be processed by trained technical staff only.

The kit 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 and incubated test strips 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

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:

After manual loading of the strips and reagent cartridges, the incubation and washing steps of the procedure are automatically processed by the BDI which ensures an efficient circulation of fluids over the strips by continuously agitating them up and down in the wells of ready-to-use reagent cartridges. The whole test procedure is run at room temperature.

STRIPS description:

The reactive (front) side of the strips is coated with antigens which appear as 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 a strip number and a 2-dimensional square barcode for traceability of the strips after removal from the BDI at the end of the test.



The non-reactive (back) side of the strips displays both alphanumeric and bar-coded information for identification of the strip type and lot number by the BDI.



The 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: (see image on page 1)

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.

STRIPS/CARTRIDGES associations

The strips and cartridges of a same test kit share the same lot number and are dedicated to be associated in lot-specific pairs. Do not associate, in a same pair, a strip and a cartridge with different lot numbers as this will be detected as an invalid setting by the BDI and will stop the process.

As far as each strip/cartridge pair is valid, the BDI can process strips/cartridges associations of different kits; However, only kits having the same protocol number (same incubation time and sequence) can be processed together in one same run (please refer to the protocol number indicated under the kit reference at the top of first page).

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 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.

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 backward 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 reads the barcodes of the strips → after complete barcode reading, **"Load reagent"** is displayed on the touch screen.

Note:

In case of failure to read one or more strip barcode(s) (flashing LED at the unread position), please refer to the Manual of Use of the BDI.

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 barcodes of the cartridges and checks the correct association with the strips → after complete barcode reading, the number of strips (validated strips/cartridges associations) is displayed on the screen.

Note:

In case of failure to read one or more cartridge barcode(s), or in case of detection of a wrong strip/cartridge association (flashing LED at the corresponding position), 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 xx**).
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 pipetting 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 steps sequence of the protocol.

8.2 Test processing (Protocol 2)

Step	Description	Processing time
01.	The strips are incubated into the 1 st well of the cartridge (<i>Diluent Buffer</i>). 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 2 nd well of the cartridge (<i>Wash Buffer</i>)	2 min
03.	The clamp moves forwards and the strips are incubated into the 3 rd well of the cartridge (<i>Wash Buffer</i>)	2 min
04.	The clamp moves forwards and the strips are incubated into the 6 th well of the cartridge (<i>Wash Buffer</i>)	2 min
05.	The clamp moves backwards and the strips are incubated into the 5 th well of the cartridge (<i>Conjugate</i>)	10 min
06.	The clamp moves backwards and the strips are incubated into the 4 th well of the cartridge (<i>Wash Buffer</i>)	2 min
07.	The clamp moves backwards and the strips are incubated into the 3 rd well of the cartridge (<i>Wash Buffer</i>)	2 min
08.	The clamp moves backwards and the strips are incubated into the 2 nd well of the cartridge (<i>Wash Buffer</i>)	2 min
09.	The clamp moves forwards and the strips are incubated into the 7 th well of the cartridge (<i>Substrate</i>)	10 min
10.	The clamp moves backwards and the strips are incubated into the 6 th well of the cartridge (<i>Wash Buffer</i>)	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

A visual evaluation of the results can be performed, however the use of the Dr DOT Software and Scanning system is generally recommended for more precise and semi-quantitative interpretation.

More information on Dr DOT is available via your distributor or via our website www.d-tek.be

9.1 Visual interpretation:

1. Remove the clamp from the BDI and unload the strips from the clamp.
2. Place the strips with reactive face up onto the marked fields of the Diver Interpretation Template provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
3. The first upper dot (Positive Control) must be positive for all patients.

Only a clearly coloured Positive Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded. If the first upper dot is not coloured, the test has failed and cannot be interpreted further.

4. Compare the specific antigen dots to the Negative Control Dot (which always is the last bottom dot). The colour intensity of the Antigen dots is directly proportional to the titer of the specific antibody in the patient sample.

The colour intensity of the Negative Control Dot may vary depending on the sample characteristics. If the sample is free of interfering substances the Negative Control Dot may be even close to uncoloured. In contrast, a highly coloured Negative Control Dot indicates a high rate of unspecific binding in the sample.

POSITIVE RESULT:

A sample is positive for a specific antibody if the colour intensity of the corresponding Antigen dot is higher than the intensity of the Negative Control Dot.

NEGATIVE RESULT:

A sample is negative for a specific antibody if the colour intensity of corresponding Antigen dot is lower than or equal to the intensity of the Negative Control Dot.

NB: A weak coloration of an antigen dot, when close to the colour intensity of the Negative Control Dot may be difficult to differentiate by visual inspection only. In such cases, it is recommended to use Dr DOT software and scanning system (see 9.2) and refer to the corresponding instructions for more accurate interpretation.

9.2 Use of Dr DOT Software and Scanning system

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

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 were within the specified range and standard deviations were less than 10 %
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.

Sci-70		CENP-A		CENP-B		PM-Sci 100					
	+	-		+	-		+	-			
+	true positive 13	false positive 0	+	true positive /	false positive /	+	true positive 10	false positive 0			
-	false negative 0	true negative 91	-	false negative /	true negative /	-	false negative 0	true negative 24			
	Sensitivity	100%		Sensitivity	- %		Sensitivity	100%			
	Specificity	100%		Specificity	- %		Specificity	100%			
				No reference method !							
PM-Sci 75		Ku		RNA-Polymerase III		U1-RNP					
	+	-		+	-		+	-			
+	true positive /	false positive /	+	true positive 22	false positive 0	+	true positive 43	false positive 2			
-	false negative /	true negative /	-	false negative 0	true negative 50	-	false negative 0	true negative 93			
	Sensitivity	- %		Sensitivity	100%		Sensitivity	100%			
	Specificity	- %		Specificity	100 %		Specificity	98%			
				No reference method !							

For the antigens in grey, since no clearly characterized sample nor reference method is currently available, no values can reasonably be claimed.

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 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.** 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.