

## Chromatin<sup>4</sup> IgG

Order Code: CHR4D-24

### 1. INTENDED USE

BlueDot Chromatin<sup>4</sup> IgG is an Immunodot kit intended for the detection in human sera of IgG autoantibodies against Nucleosome, dsDNA, Histones and DFS-70 antigens.

This kit is intended to confirm and differentiate aspects in homogenous patterns obtained by Immunofluorescence, the screening and reference method in autoimmunity; the kit is intended as an aid in the diagnosis of the following autoimmune diseases (see 11.3 *Clinical sensitivity and specificity* for the link with each autoantibody): Systemic Lupus Erythematosus (SLE), Drug-induced Lupus (DIL), Rheumatoid arthritis, Juvenile Idiopathic Arthritis (JIA), Sjögren's Syndrome (SjS), Myasthenia Gravis, Autoimmune Hepatitis.

The test is intended for a large, routine population.

This kit is strictly reserved for professional use.

It can only be used manually on a platform shaker or in an open automated immunodot processing system, programmed according to the pipetting scheme described in point 9.2.

### 2. PRINCIPLE OF THE TEST

The test is based on the principle of an Enzyme Immunoassay. The strips are composed of a membrane fixed on a specific plastic support. 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.

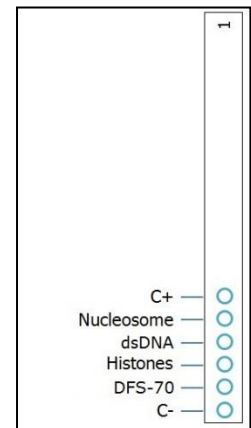
### 3. KIT CONTENTS

**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.**

#### 3.1 COMPONENTS

<u>TO BE DILUTED:</u>	<b>(10 x) Wash buffer</b>	<b>1 x 40 ml - 10x concentrated</b> (colourless) <i>Contains: H<sub>2</sub>O • TBS • NaCl • Tween • MIT</i>
<u>READY TO USE:</u>	<b>Dot strips</b>	<b>24 units</b> 6 dots each: 1 negative control (C-) 4 antigens 1 positive control (C+)
	<b>Diluent buffer</b>	<b>1 x 40 ml</b> (yellow) <i>Contains: H<sub>2</sub>O • TBS • NaCl • Tween • BSA • MIT • Dye</i>
	<b>Conjugate</b>	<b>1 x 40 ml</b> (red) <i>Contains: H<sub>2</sub>O • TBS • NaCl • KCl • MgCl<sub>2</sub> • AP-conjugated goat anti-human IgG • MIT • Dye</i>
	<b>Substrate</b>	<b>1 x 40 ml</b> (brown bottle, pale yellow solution) <i>Contains: H<sub>2</sub>O • NaN<sub>3</sub> (0.05 %) • MgCl<sub>2</sub> • TBS • NBT • BCIP • NBT Stabilizer</i>
	<b>Incubation trays</b>	<b>3 units</b> with 8 wells for incubation



*Abbreviations in alphabetic order:*

AP = Alkaline Phosphatase; BCIP = Bromo-Chloro-Indolyl-Phosphate; BSA = Bovine Serum Albumin; KCl = Potassium Chloride; MgCl<sub>2</sub> = Magnesium Chloride; MIT = MethylIsoThiazolone (preservative); NaCl = Sodium Chloride; NaN<sub>3</sub> = Sodium Azide; NBT = NitroBlue Tetrazolium; TBS = Tris Buffer Saline

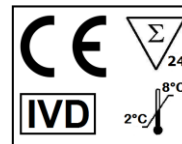
For more information on the composition and concentration of the active ingredients used, please refer to the MSDS available on request or on [www.d-tek.be](http://www.d-tek.be).

#### 3.2 Antigens used

Nucleosome	dsDNA wrapped around a core histones octamer. Heterogenous mixture of pure native poly-nucleosomes composed of about 7 to 28 mono-nucleosomes. Contains Histones H2a, H2b, H3-H4 and traces of H1 (purified from bovine thymus chromatin)
dsDNA	double-stranded dsDNA (purified from bovine thymus)
Histones	Mixture of H1, H2a, H2b, H3-H4 (purified from bovine thymus)
DFS-70	Lens Epithelium Derived Growth Factor ( <b>D</b> ense <b>F</b> ine <b>S</b> peckles protein <b>70</b> kD) (recombinant, human, expressed in E.coli)



We Apply Science



IFU – Instructions for Use  
CHR4D-24/p. 2 of 8

#### 4. MATERIAL REQUIRED BUT NOT PROVIDED

Platform shaker / Micropipettes / Timer / Graduated cylinder / Distilled or deionised water / Tweezers / Absorbent and/or filter paper.

#### 5. STORAGE

The reconstituted Wash Solution is stable for at least one month at 2-8°C. Reagents and strips can be stored at 2-8°C until the expiry date indicated on each vial or tube.

Place unused strips back into the provided tube, seal it and store at 2-8°C. Chromogen/Substrate (NBT/BCIP) shall be stored at 2-8°C.

#### 6. SAFETY PRECAUTIONS

1. All reagents are for in vitro diagnostic and professional use only. The test kit should be processed by trained technical staff only.
2. The reagents in the kit are considered as not dangerous, as the concentrations of potentially dangerous chemicals are below the thresholds specified by European regulations (see MSDS). Nevertheless, the product contains preservatives which may have (in their given concentration), slightly polluting properties or causing skin sensitization. Therefore, contact with the skin, eyes or mucous membranes should be avoided. As with any chemical containing specific hazards, the product/components of the product should only be handled by qualified personnel and with the necessary precautions.
3. Patient samples should be handled as if they were capable of transmitting infectious diseases; they therefore require suitable protection (gloves, laboratory coat, goggles). In any case, GLP should be applied with all the general or individual safety rules in force.
4. Waste disposal: Patient samples, incubated test strips and used reagent vials should be handled as infectious waste. The boxes and other containers do not need to be collected separately, unless stated otherwise in official regulations.

#### 7. RECOMMENDATIONS

1. D-tek and its authorized distributors cannot be held responsible for damages caused indirectly or due to: a change or modification in the indicated procedure, an improper use of the kit and / or the use of an incomplete or damaged kit. The use of this kit is reserved for qualified technical personnel only.
2. D-tek's responsibility is limited in all cases to the replacement of the kit.
3. In the event of a serious incident (injury, deterioration in health, or death) with this IVD device, please report it immediately to the manufacturer (see address below) and to the competent authority in your country.

#### 8. SAMPLE COLLECTION, HANDLING AND STORAGE

The test should preferably be used on recently collected samples. Sera with particles should be centrifuged at low speed. Blood samples should be collected in dry tubes or tubes containing EDTA or heparin. After separation, the serum samples should be used immediately or aliquoted and stored at 2-8 ° C (for storage for a few days) or frozen at -20°C (for longer storage periods). Repeated freezing/ thawing cycles of the samples must be avoided.

#### 9. ASSAY PROCEDURE

##### **BASIC INFORMATION, HANDLING AND TIPS:**

The dots are precoloured blue on the strips, ensuring that all antigens have been dotted correctly onto the membrane. This blue coloration disappears during the first step of the incubation. During incubation with the wash buffer, a faint pink background coloration appears on the membrane and disappears upon drying at the end of the procedure.

During the procedure, agitation of the incubation tray is necessary to ensure efficient circulation of fluids over the membrane. A Rocking platform is the shaker of choice. Be sure to adjust the movement of the shaker in such a way that no spilling of solutions or cross-contamination between the wells can occur.

After each filling of the wells with solution, agitate manually the incubation tray until the strips are completely immersed in order to remove air bubbles which may be trapped under the strip. Alternatively, floating strips may be forced into the solution by pushing down (with tweezers or pipette tip) on the upper part of the strip (plastic label zone).

**Avoid touching** the membrane zone of the strip with fingers, tweezers or pipette tips. Always use the plastic label zone for handling or manipulation. The whole procedure has to be run **at room temperature**.

##### **Description of the CONTROLS:**

The **Positive Control or RC (Reaction Control)** consists of a protein fixing all the immunoglobulins present in the test sample. If the test has been carried out correctly, this control will show a colouring at the end of the test (with an intensity depending on the effective concentration of immunoglobulins in the sample).

The absence of any colouring of this dot at the end of the test may indicate that the sample has not been pipetted on the strip (see 10.4 *Troubleshooting*).

The **Negative Control or CO (Cut-Off Control)** consists of a protein reacting with the enzymatic substrate and with certain constituent elements of the tested sample. If the test has been carried out correctly, this control is coloured at the end of the test, with a signal depending on the kinetics of the substrate and the characteristics of the sample. The intensity of this control serves as a threshold value for the final interpretation of the results (see 10 *INTERPRETATION OF RESULTS*).



### 9.1 Reagents preparation

1. Allow all components to equilibrate at room temperature before use.
2. **Dilute** the concentrated **Wash Buffer 10x** with **distilled water**.  
*Prepare 15 ml diluted Wash buffer per strip tested*  
*Example: 1,5 ml concentrated wash buffer + 13,5 ml distilled water for one strip.*

### 9.2 Pipetting flow chart

1. **Place** one **strip** per patient into the wells, blue dots **facing up**.
2. Add **2 ml Wash Buffer** per well. **Incubate** (shake) **for 10 min**.  
*Upon correct incubation, the blue coloration of the dots completely disappears.*  
*If not prolong the procedure until the colour of the dots fades completely.*
3. **Discard** solution from the wells.  
*Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.*
4. Add **1,5 ml Sample Diluent** per well.
5. Add **10 µl patient sample** per well. **Incubate** (shake) **for 30 min**.  
*Avoid touching the membrane with the pipette tip. Preferentially dispense the sample into the solution over the upper part of the strip (plastic label zone).*  
*Note: Steps 4 and 5 can be combined by pre-diluting the sample in a glass or plastic tube (1,5 ml diluent + 10 µl patient sample). Mix (Add to the well)*
6. **Discard** solution from the wells.  
*Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.*
7. **Wash 3 x 3 minutes** with **1,5 ml Wash Buffer** per well (shake).  
*Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper*
8. Add **1,5 ml Conjugate** per well. **Incubate** (shake) **for 30 min**.
9. **Discard** solution from the wells.  
*Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper*
10. **Wash 3 x 3 min.** with **1,5 ml Wash Buffer** (shake)  
*Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.*
11. Add **1,5 ml Substrate** per well. **Incubate** (shake) **for 10 min**.
12. **Discard** solution from the wells.  
*Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.*
13. **Wash 1 x 3 min.** with **1,5 ml Wash solution** per well to stop the reaction.
14. **Collect** the strips from the wells and allow them to dry for 30 minutes on absorbent paper. The interpretation has to be done in the 24 hours following the test processing.

## 10. INTERPRETATION OF RESULTS

A visual (qualitative) interpretation of the results of BlueDOT kits is possible, however the use of the BlueScan scanner and the DrDOT software is generally recommended for more precision and a semi-quantitative interpretation.

### 10.1. Qualitative Interpretation

1. Peel off the cover of the adhesive on the back side of each strip and attach strips dots face up onto the marked fields of the interpretation sheet provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
2. The first upper dot (**Positive Control Dot**) 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.
3. 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.

### 10.2 Use of Dr DOT Software and Scanning system (material needed: BlueDiver Clamp, empty stripholders)

The BlueScan scanner is an especially designed system for the reading of D-tek immunodot strips. It allows precise and easy insertion of test strips.

The Dr DOT software allows a semi-quantification of results. Based on the image obtained, each result will be quantified in grayscale value and compared to the reference scale integrated in the BlueScan Cover.

These grayscale intensities will be transformed and displayed in arbitrary units (AU, from 0 to 100) based on the intensities of the controls (RC and CO, see point 9) present on the strip, according to the following conversion formula:

$$\text{Result of antigen X (AU)} = \frac{\text{Grayscale intensity of antigen X} - \text{Grayscale intensity of CO}}{\text{Grayscale intensity of RC} - \text{Grayscale intensity of CO}} * 100$$

1. Prepare a BlueDiver Clamp and load it with as many empty stripholders as there are strips to analyse. Carefully insert a strip into each stripholder, RC showing upwards.
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.
4. The software semi-quantifies the results, and the interpretation of the obtained values is as follows

Dr DOT arbitrary unit (AU)	Interpretation
< 5	Negative
5 – 10	Equivocal (*)
>10	Positive

For detailed information about the BlueScan and Dr DOT software please refer to the Manual of Use of your Dr DOT software

### 10.3 Important recommendations for the interpretation of results

1. D-tek's kits constitute a diagnostic aid. In consequence, no diagnosis can be established solely on the basis of our kits. The results should always be interpreted by taking into account the clinical examination, the patient's history and the results obtained by other methods.  
No single technique can rule out the possibility of false positive or false negative results. With this in mind, an indirect immunofluorescence test should, as far as possible, be carried out prior to the use of a BlueDot kit (immunofluorescence being recognized as a reference method in autoimmunity).
2. The intensity of a result is not necessarily related to the degree of intensity of the disease, but rather to the level of antibodies detected.
3. Low titers of auto-antibodies may occur in healthy patients. For this reason, low positive results (close to the CO, between 5 and 10 DrDot AU), although valid, should be considered equivocal. In such cases, the retesting of the patient, preferably by using a new sample, is recommended. If the result remains equivocal on retesting, other diagnostic tests and/or clinical information should be used to help determine the autoimmune status of the patient.
4. For various reasons, and under certain conditions, the kit may show a defect in performance (see 10.4 *Troubleshooting*). In such cases, the results are not valid and cannot be interpreted. It is recommended to repeat the test. If the error persists, please contact your distributor.

### 10.4 Troubleshooting

Problem	Possible causes + Action
Discrepancy of results as compared to a reference method	<ul style="list-style-type: none"> <li>- Use                             <ul style="list-style-type: none"> <li>- incorrect pipetting of serum</li> <li>- incorrect volume dispensed</li> <li>- erroneous visual interpretation</li> <li>- erroneous DrDot reading</li> <li>→ repeat the test</li> </ul> </li> <li>- Material                             <ul style="list-style-type: none"> <li>- Interfering substance in the sample</li> <li>→ repeat the test and confirm by other methods</li> </ul> </li> <li>- Method                             <ul style="list-style-type: none"> <li>- intrinsic performance of the kit (see 11.2 <i>Analytical sensitivity and specificity</i>)</li> <li>- expired kit</li> <li>- stability problem</li> </ul> </li> </ul> <p><b>Please contact your distributor for any further technical support requests.</b></p>
Different results in the same batch or between several batches -	<ul style="list-style-type: none"> <li>- Use                             <ul style="list-style-type: none"> <li>- incorrect pipetting of serum</li> <li>- incorrect volume dispensed</li> <li>- erroneous visual interpretation or</li> <li>- bad DrDot reading</li> <li>→ repeat the test</li> </ul> </li> <li>- Method                             <ul style="list-style-type: none"> <li>- intrinsic performance of the kit (see 11.1 <i>Repeatability and Reproducibility</i>)</li> </ul> </li> </ul>

Contamination between neighbouring strips	- Use incorrect pipetting of serum → repeat the test
RC absent or weak	- Use Serum not pipetted at all → repeat the test - Patient with immunoglobulin deficiency → repeat the test to confirm patient status - Damaged reagents → check the integrity of the reagents → contact your supplier if you suspect a problem - Spot not on the strip → count the number of dots on the strip; if not correct, contact your supplier
CO absent	- damaged reagents → check the integrity of the reagents, contact your distributor if you suspect a problem - Spot absent from the strip → count the number of spots present on the strip, contact your distributor in case of incorrect number
Non-specific bindings / high background / high CO value	Suspected presence of a contaminant or an interfering substance in the patient sample → repeat the test and confirm through another method  <b>Please contact your distributor for any further technical support requests.</b>
Strips not correctly labelled	Manufacturing problem → please contact your distributor
Kit content incorrect	Manufacturing problem → please contact your distributor

**NOTE:**

The major residual risks of the kit, as given in the risk analysis of the kit at the end of design (after mitigation), are the following:

- 1) Risk of false results based on a pipetting error (bad serum)
- 2) Risk of false results based on an interfering substance contained in the sample

**11. PERFORMANCES**

**11.1 Repeatability and Reproducibility**

Reference samples were tested for each antibody in successive statistically representative series, both in the same test as in different tests and between different batches in order to calculate the intra-assay, inter-assay and inter-lot variations respectively. In all the cases, the variations in colour intensity were within the following expected limits:  
 CV ≤ 10% for intra-assay runs  
 CV ≤ 15% for inter-assay runs  
 CV ≤ 20% for inter-lot runs

**11.2 Analytical 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.

<b>Nucleosome</b>		<b>dsDNA</b>		<b>Histones</b>		<b>DFS-70</b>	
+		+		<b>No comparative data available</b>		+	
True positive <b>36</b>	False positive <b>1</b>	True positive <b>52</b>	False positive <b>2</b>			True positive <b>24</b>	False positive <b>1</b>
False negative <b>18</b>	True negative <b>49</b>	False negative <b>1</b>	True negative <b>105</b>			False negative <b>0</b>	True negative <b>134</b>
<b>Sensitivity</b>	$\frac{36}{54} = 67\%$	<b>Sensitivity</b>	$\frac{52}{53} = 98\%$			<b>Sensitivity</b>	$\frac{24}{24} = 100\%$
<b>Specificity</b>	$\frac{49}{50} = 98\%$	<b>Specificity</b>	$\frac{105}{107} = 98\%$	<b>Specificity</b>	$\frac{134}{135} = 99\%$		

### 11.3 Clinical sensitivity and specificity

Anti-nucleosomes	<ul style="list-style-type: none"> <li>- Diagnostic marker of → Systemic lupus erythematosus (SLE)</li> <li>- Sensitivity for SLE is between 56-90 %.</li> <li>- Can be detected at an early stage of disease</li> <li>- Can be detected in patients with → drug-induced lupus (DIL)</li> </ul>
Anti-dsDNA	<ul style="list-style-type: none"> <li>- Diagnostic marker (ACR and SLICC criterion) of → Systemic lupus erythematosus (SLE)</li> <li>- frequently detected: <ul style="list-style-type: none"> <li>In &gt;95 % in active SLE with renal involvement,</li> <li>In &gt;50-70% in active SLE without renal involvement</li> <li>In &lt;40% in inactive SLE</li> </ul> </li> <li>- Prognostic marker associated with the severity of → SLE.</li> <li>- can be detected by EIA (1-12%) in patients with → rheumatoid arthritis, → juvenile idiopathic arthritis, → Sjögren's syndrome, → systemic sclerosis, myasthenia gravis, autoimmune hepatitis, uveitis, → drug-induced lupus-like syndromes and → various infectious diseases</li> </ul>
Anti-histones	<ul style="list-style-type: none"> <li>- can be detected in various rheumatic disorders: → Systemic lupus erythematosus (SLE; 50-80%), → drug-induced lupus (DIL; 92-95%), → rheumatoid arthritis (RA; 11%) and rheumatoid arthritis vasculitis (up to 75%), → Felty's syndrome (up to 79%), → juvenile idiopathic arthritis (JIA; up to 51%), systemic sclerosis (SSc; up to 30%), ANA positive undifferentiated connective tissue diseases (up to 90%), primary biliary cirrhosis (up to 55%), autoimmune hepatitis (up to 35%)</li> <li>- The detection of high AHA (anti-histones) titers in the absence of SLE marker antibodies is characteristic of drug induced lupus (DIL)</li> </ul>
Anti-DFS70	<ul style="list-style-type: none"> <li>- The highest frequencies of DFS70 antibodies have been reported in patients with → Vogt-Koyanagi-Harada syndrome (77%), → atopic dermatitis (30-71%) and → asthma (16%).</li> <li>- detected in 5-11% of healthy blood donors.</li> <li>- The presence of isolated anti-DFS70 antibodies could be used as a biomarker to exclude the diagnosis of systemic autoimmune rheumatic diseases (SARD)</li> </ul>

Reference: "Autoantibodies in Systemic Autoimmune Diseases, A Diagnostic Reference", volume 2, third edition – 2015  
Karsten Conrad, Werner Schössler, Falk Hiepe, Marvin J. Fritzler.

### 12. TEST LIMITATIONS

- The main known interfering substances were tested on each biomarker of the present kit. For each concentration of interfering substance tested, the difference between the result of the sample without the interfering substance and the result obtained in the presence of the interfering substance did not exceed 15%.

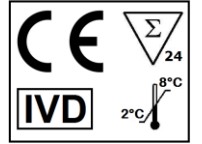
Interfering substance	Maximum Concentration	Intermediate Concentration	Minimum Concentration	Difference <15%
Bilirubin	100 mg/dL	50 mg/dL	25 mg/dL	Yes
Haemoglobin	200 mg/dL	100 mg/dL	50 mg/dL	Yes
Cholesterol	224.3 mg/dL	112 mg/dL	56 mg/dL	Yes
Rheumatoid factor IgM	~500IU/ml	~300IU/ml	~100IU/ml	Yes

Note: It is impossible to test all the possible interfering substances described in the literature. Other interferences, amongst others drug-induced interferences, are possible.

- There are no known cross-reactivities for the biomarkers in this kit.
- The results obtained with this confirmatory test are dependent on the intrinsic performance of the kit and must be considered as an aid to the final diagnosis, taking into account the results obtained by a reference technique and the clinical data of the patient.



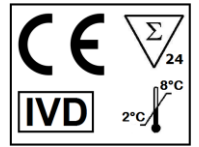
We Apply Science



IFU – Instructions for Use  
CHR4D-24/p. 7 of 8



We Apply Science



IFU – Instructions for Use  
CHR4D-24/p. 8 of 8

Version A  
Last revision: 02/2020

