GenVinSet

HLA Behçet v5

Instructions for Use

Kit for the detection of the HLA-B*51/52 group of alleles

Product code GVS-B505-48 (48 test)
GVS-B505-24 (24 test)

Store from –18 to –30ºC

For in vitro Diagnostic Use

Rev02/16-02-2021

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Intended use

Genvinset® HLA Behçet v5 is a kit for the determination of the HLA-B*51/52 group of alleles by Real time PCR using Taqman® probes technology.

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Summary and explanation

Behçet's Disease (BD) is a form of vasculitis that manifests with mouth ulcers, orogenital ulcers, uveitis, skin inflammation, enterocolitis and inflammation in other organs (1,2).

Despite being a worldwide-distributed illness, BD is more frequent in the area that extends from East Asia to the Mediterranean basin (1, 3). It is also relatively common in countries such as Turkey and Japan (2).

According to various scientific studies, there are several genetic factors that partially determine the susceptibility to develop the illness (1, 3, 4). More specifically, it is known that BD is associated with the HLA-B*5 antigen, belonging to the MCH (Major Histocompatibility Complex) (4), encoded by the HLA-B*51/52 group of alleles (5-7). Therefore, the HLA-B*5 antigen is a risk factor strongly associated with BD.

The nature of the clinical manifestations of BD established the question of whether the HLA-B*5 antigen may have a modulating effect on the expresion of the disease. Results from different studies suggest that HLA-B*5 positive patients more frequently develop symptoms that affect the central nervous system or organs such as the eye (1), whereas HLA-B*5 negative patients more frequently develop thrombophlebitis (8).

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Procedure principles

The detection method used by Genvinset® is based on the Real Time PCR technology, monitored with Taqman® probes.

The Genvinset® HLA Behçet v5 kit allows for the determination of the HLA-B*51/52(*) group of alleles and a control gene (ß-Globin), used to verify the results of the assay.

Each pair of primers is complementary to two DNA sequences, located in cis, thus making of the current kit a high resolution technique with high sensitivity, specificity and reproducibility.

(*) See section 'Procedure Limitations'.

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Kit contents

Product code GVS-B505-48 (48 tests)

- GVS-B5v5-PM: 2 vials x 220 µL Primer Mix (PM)
- GVS-B5v5-MM: 2 viales x 276 µL Master Mix (MM)
- GVS-B5v5-C+: 1 vial x 10 µL Positive Control (C+)
- GVS-RB: 1 vial x 100 µL Reaction Blank (RB)

Product code GVS-B505-24 (24 tests)

- GVS-B5v5-PM: 1 vial x 220 µL Primer Mix (PM)
- GVS-B5v5-MM: 1 vial x 276 µL Master Mix (MM)
- GVS-B5v5-C+: 1 vial x 10 µL Positive Control (C+)
- GVS-RB: 1 vial x 100 µL Reaction Blank (RB)

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Kit storage

In order to ensure a proper performance, reagents should be stored from -18ºC to -30ºC until their expiration date, indicated on the label of the vial. Do not perform more than 3 freeze/thaw cycles to the Primer Mix (GVS-B5v5-PM) and Master Mix (GVS-B5v5-MM) vials, as this could decrease the sensitivity of the assay and impair results.

Due to the reagent's photo sensitive nature, avoid continuous exposure to light.

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Materials required but not supplied

General

- Gloves
- Lab coat

Consumables

- Filter tips (P200, P100 and P10)
- 1,5 mL autoclaved tubes
- q-PCR instrument specific reagents (in the case of using Rotor-Gene® Q, only 0.1 mL tubes are allowed).

Equipment

- q-PCR instrument, with FAM and HEX/VIC detection channels. The following devices have been validated:
    - Rotor-Gene® Q, Qiagen®
    - DTlite, DNA Technologies
    - QTower, Analytic Jena
    - 7500 Real-Time PCR System, Applied Biosystems™
- Vortex mixer
- Pipettes (P200, P100 & P10)

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Sample collection and preparation

This test should only be performed with complete blood samples treated with EDTA anti coagulation agents or citrate. Heparin can interfere with the PCR process and should not be used in this procedure.

The technique is compatible with any conventional DNA extraction system. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

Caution

All biological and blood samples should be treated as potencially infectious. All the corresponding basic (universal) precautions should be taken. Samples should always be handled wearing the appropriate personal protection equipment.

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Procedures

A) PCR setup

Precautions

- Thaw all components of the kit before starting the assay, mix and centrifuge them.
- Work on ice or over a cool block.
- The PCR should be assembled in the Pre-PCR area.
- Use only filtered tips and autoclaved tubes 1.5 mL.
- Use gloves and lab coat at all times.
- In each session it is recommended to test the contamination control (Reaction Blank) and the Positive Control (B*51/52) included in the kit, as well as a B*51/52 negative sample.

1. Thaw the samples. Vortex (or gently mix with the fingers).

2. Prepare the following mix for n+1 samples:

	Vol. per sample (µL)
Master Mix	10
Primer Mix	8

3. Pipette 18 µL of the mix in each well. Then add 2 µL of DNA, Positive Control or Reaction Blank in the corresponding well.

4. Seal the plate or the tubes. Perform a brief spin to ensure that all the volume settles to the bottom of the tube.

5. Place the plate in the thermal cycler and start the following amplification program.

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B) Thermal cycler configuration

1. Set up the following amplification program:

	Cycle Number	Temperature (ºC)	Time (mm:ss)	Ramp (%)	Analysis
Denaturation	1	95	05:00	100	X
Cycles	40	95	00:15	100	X
		66	01:00	100	Single
Cooling	1	15	∞	100	X

2. Set up the reading channels as indicated below:

The emitted fluorescence must be read in the FAM (495-520 nm) and HEX (535-554 nm) channels. Both fluorescences should be detected in every well (biplex reaction).

NOTE - Set the following configuration in the Rotor-Gene® Q software:

a. Open the Rotor-Gene® Q – Pure Detection software. Select the tab "Advanced" within the "New Run" window and click on "New".

b. Choose the "72-Well Rotor" format and check the box "Locking Ring Attached". Then, click on "Next".

c. Set the "Reaction Volume" at 20 µL and identify the operator and the samples.

d. Click on "Edit Profile" and set up the amplification program. Set the 60 sec step at 66 ºC, and click on "Acquiring to Cycling A". Set "Green" and "Yellow" as the fluorescence acquisition channels. Click on "OK" to accept and close the "Edit Profile" window.

e. Click on "Gain Optimisation" in the "Run New Wizard" dialog box to open the "Auto-Gain Optimization Setup" window. Scroll down the "Channel Settings" menu and select "Acquiring Channels". Then click on "Add". In the window "Auto-Gain Optimization Channel Settings", set the following parameters for each channel ("Green" and "Yellow"):

- Tube position = 1
- Target Sample Range: 5 FI up to 10 FI
- Acceptable Gain Range: -10 to 10

f. Check the box "Perform Optimisation Before 1st Acquisition" and click on "Close".

g. Click on "Next" and then on "Start Run" in the "New Run Wizard" window.

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Results

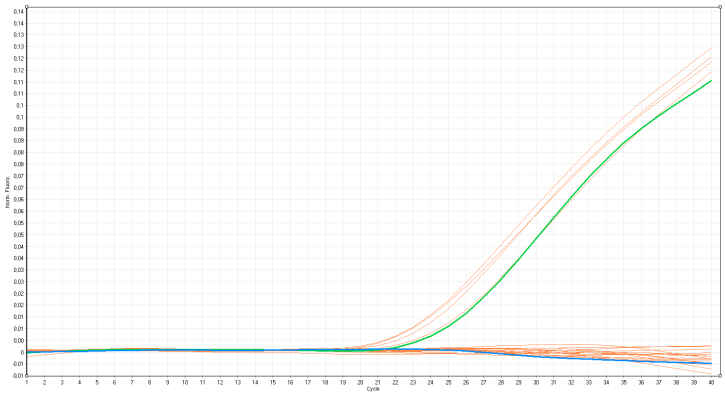
The Genvinset® HLA Behçet v5 kit constitutes a qualitative technique to determine the presence or absence of the HLA-B*51/52 group of alleles.

It is not necessary to select any passive reference.

The results of this technique can be obtained as follows:

HLA-B*51/52 results

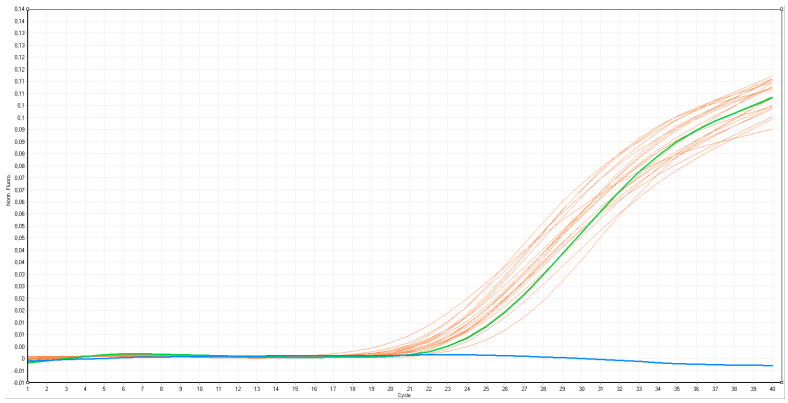
Selecting the "Green" channel (FAM) in the Amplification Plot, a graphic similar to the following one can be displayed:



Samples generating an amplification curve are to be considered as B*51/52 positive and can be identified by a numeric value called Crossing Point (Cp), which corresponds to the cycle in which fluorescence can be detected.

ß -globin results

Selecting the "Yellow" channel (HEX/VIC) in the Amplification Plot, a graphic similar to the following one can be displayed:



Samples generating an amplification curve are positive for internal control (ß-globin).

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Quality control

Due to the qualitative nature of this test, it will not be necessary to perform a calibration.

It is recommended to perform a contamination control by replacing the DNA for the negative control supplied in the kit and a positive control (HLA-B*51/52 positive sample).

The following criteria have to be taken into account in order to validate an assay:

- The Reaction Blank should report negative results in both "Green" and "Yellow" channels. An amplification curve with a Cp>35 value should be considered as negative. A Cp<35 value is associated with a contamination in the Reaction Blank and, therefore, all results from the experiment should be discarded.
- A positive control sample must render positive results for both B*51/52 and for ß-globin.
- DNA samples should always be positive for ß-globin. (Cp<35).
- DNA samples generating results with Cp>35 for ß-globin and/or B*51/52 must be considered as doubtful and must be re-tested by performing a new extraction of DNA.

The assay must be performed according to the kit recommendations, as well as other quality control procedures that comply with local, federal and/or certifying agencies specifications.

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Specific operation data

1. Analytical specificity

The alignment of primers and probes in the most common HLA database (IMGT-HLA) has revealed the absence of non-specific bindings. No cross-reaction phenomena with genomic DNA have been reported.

2. Analytical sensitivity

A serial dilution assay was performed using two DNA samples (one B*51 positive and one B*51/52 negative), at a concentration of 35,8 and 25,7 ng/µL, respectively. Both were obtained with a conventional DNA extraction system. The following results for the analytical sensitivity of the test were obtained:

- DNA sample obtained by conventional extraction system: Detection Limit = 0,034 ng/µL (*)

  (*) Cp < 35

3. Diagnostic sensitivity and specificity

In three studies of human genetic DNA, one performed in an internal laboratory and the other two in external laboratories, a total of 157 samples were tested with the Genvinset® HLA Behçet v5 kit. The samples had been previously typed by SSO (Sequence Specific Oligonucleotides).

All the 157 tested samples were validated (positive amplification of the ß-Globin control gene). Among those, 44 were found to be B*51/52 positive.

	Genvinset® HLA Behçet v5		
SSO	Samples	B*51/52 +	B*51/52 -
	B*51/52 +	44	0
	B*51/52 -	0	113

There is a 100% match in the results obtained with the Genvinset® HLA Behçet v5 kit and the previously obtained genotype with the SSO technology.

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HLA-B*51/52 alleles (IMGT-HLA 3.43.0) detected by Genvinset® HLA Behçet v5

B*51:01:01:01	B*51:01:01:23	B*51:01:01:45	B*51:01:01:67
B*51:01:01:02	B*51:01:01:24	B*51:01:01:46	B*51:01:01:68
B*51:01:01:03	B*51:01:01:25	B*51:01:01:47	B*51:01:01:69
B*51:01:01:04	B*51:01:01:26	B*51:01:01:48	B*51:01:01:70
B*51:01:01:05	B*51:01:01:27	B*51:01:01:49	B*51:01:02:01
B*51:01:01:06	B*51:01:01:28	B*51:01:01:50	B*51:01:02:02
B*51:01:01:07	B*51:01:01:29	B*51:01:01:51	B*51:01:05
B*51:01:01:08	B*51:01:01:30	B*51:01:01:52	B*51:01:07
B*51:01:01:09	B*51:01:01:31	B*51:01:01:53	B*51:01:09
B*51:01:01:10	B*51:01:01:32	B*51:01:01:54	B*51:01:42
B*51:01:01:11	B*51:01:01:33	B*51:01:01:55	B*51:01:55
B*51:01:01:12	B*51:01:01:34	B*51:01:01:56	B*51:01:56
B*51:01:01:13	B*51:01:01:35	B*51:01:01:57	B*51:01:57
B*51:01:01:14	B*51:01:01:36	B*51:01:01:58	B*51:01:58
B*51:01:01:15	B*51:01:01:37	B*51:01:01:59	B*51:01:59
B*51:01:01:16	B*51:01:01:38	B*51:01:01:60	B*51:01:60
B*51:01:01:17	B*51:01:01:39	B*51:01:01:61	B*51:01:61
B*51:01:01:18	B*51:01:01:40	B*51:01:01:62	B*51:01:62
B*51:01:01:19	B*51:01:01:41	B*51:01:01:63	B*51:01:63
B*51:01:01:20	B*51:01:01:42	B*51:01:01:64	B*51:01:64
B*51:01:01:21	B*51:01:01:43	B*51:01:01:65	B*51:01:65
B*51:01:01:22	B*51:01:01:44	B*51:01:01:66	B*51:01:66

- Detected allele
- ***Common allele of the CWD Catalogue***
- Non tested allele. Possible weak amplification

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HLA-B*51/52 alleles (IMGT-HLA 3.43.0) detected by Genvinset® HLA Behçet v5

B*51:01:67	B*51:02:01:03	B*51:36	B*51:219
B*51:01:68	B*51:02:02	B*51:40	B*51:224
B*51:01:69	B*51:02:06	B*51:43	B*51:230:01:01
B*51:01:70	B*51:03	B*51:50	B*51:230:01:02
B*51:01:71	***B*51:05***	B*51:65	B*51:230:01:03
B*51:01:72	***B*51:07:01***	B*51:75	B*51:232:01
B*51:01:73	***B*51:08:01:01***	B*51:78:01	B*51:233
B*51:01:74	B*51:08:01:02	B*51:83	B*51:234
B*51:01:75	B*51:08:01:03	B*51:152	B*51:235N
B*51:01:76	B*51:08:01:04	B*51:158:02	B*51:238
B*51:01:77	B*51:08:03	B*51:168	B*51:239
B*51:01:78	B*51:08:04	B*51:173Q	B*51:240
B*51:01:79	***B*51:09:01***	B*51:187	B*51:241
B*51:01:80	B*51:13:02	B*51:188	B*51:242
B*51:01:81	B*51:14	B*51:189	B*51:243
B*51:01:82	B*51:16	B*51:190	B*51:244
B*51:01:84	B*51:17	B*51:192	B*51:246
B*51:01:85	B*51:19	B*51:193	B*51:247
B*51:01:86	B*51:20	B*51:204:02	B*51:248
B*51:01:87	B*51:22	B*51:206	B*51:249
B*51:02:01:01	B*51:31	B*51:208	B*51:250
B*51:02:01:02	B*51:32	B*51:214	B*51:251

- Detected allele
- ***Common allele of the CWD Catalogue***
- Non tested allele. Possible weak amplification

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HLA-B*51/52 alleles (IMGT-HLA 3.43.0) detected by Genvinset® HLA Behçet v5

B*51:252	B*51:278	B*51:306N	B*52:01:01:03
B*51:253	B*51:279	B*51:307	B*52:01:01:04
B*51:254	B*51:280	B*51:308	B*52:01:01:05
B*51:255	B*51:282	B*51:309	B*52:01:01:06
B*51:256N	B*51:283	B*51:311	B*52:01:01:07
B*51:257	B*51:285	B*51:312	B*52:01:01:08
B*51:258	B*51:286	B*51:313N	B*52:01:01:09
B*51:259	B*51:287N	B*51:314	B*52:01:01:10
B*51:260	B*51:288	B*51:316	B*52:01:01:11
B*51:262	B*51:289	B*51:317	B*52:01:01:12
B*51:263	B*51:290	B*51:318N	B*52:01:01:13
B*51:264N	B*51:291	B*51:320	B*52:01:01:14
B*51:265	B*51:292	B*51:322	B*52:01:01:15
B*51:266	B*51:294	B*51:323	B*52:01:01:16
B*51:267	B*51:296	B*51:325N	B*52:01:01:17
B*51:268	B*51:297	B*51:326	***B*52:01:02:01***
B*51:269	B*51:298	B*51:329Q	B*52:01:02:02
B*51:270	B*51:299	B*51:330	B*52:01:02:03
B*51:271	B*51:302	B*51:331	B*52:01:02:04
B*51:272	B*51:303	B*51:332	B*52:01:02:05
B*51:275	B*51:304	***B*52:01:01:01***	B*52:01:28
B*51:277	B*51:305	B*52:01:01:02	B*52:01:30

- Detected allele
- ***Common allele of the CWD Catalogue***
- Non tested allele. Possible weak amplification

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HLA-B*51/52 alleles (IMGT-HLA 3.43.0) detected by Genvinset® HLA Behçet v5

B*52:01:31	B*52:21:01	B*52:87
B*52:01:32	B*52:31:01	B*52:89N
B*52:01:33	B*52:40	B*52:90
B*52:01:34	B*52:43	B*52:91
B*52:01:35	B*52:50	B*52:92
B*52:01:36	B*52:54	B*52:93
B*52:01:37	B*52:64	B*52:94N
B*52:01:38	B*52:68	B*52:95
B*52:01:39	B*52:71	B*52:96N
B*52:01:40	B*52:74	B*52:97
B*52:01:41	B*52:75	B*52:98
B*52:01:42	B*52:76	B*52:99
B*52:01:43	B*52:77	B*52:101
B*52:01:44	B*52:78	
B*52:01:45	B*52:79	
B*52:01:46	B*52:80	
B*52:01:47	B*52:81	
B*52:01:48	B*52:82	
B*52:02:01	B*52:83	
B*52:03	B*52:84	
B*52:04	B*52:85	
B*52:10:01	B*52:86	

- Detected allele
- ***Common allele of the CWD Catalogue***
- Non tested allele. Possible weak amplification

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Procedure limitations

- The current method allows for the detection of all HLA-B*51/52 group of alleles specified in the previous section (IMGT-HLA 3.31.0).
- All the aforementioned conditions for the setup of the PCR should be carefully controlled. Any performances that do not meet such indications, can lead to poor results.
- The presence of mutations or polymorphisms at the primer/probes annealing sites is possible and may result in the lack of allele definition. Alternative technologies could be necessary to resolve the typing.
- All Genvinset® components manipulation must be done according to general lab best practices and be adjusted to local regulations, like the EFI standard (European Federation of Immunogenetics).
- The q-PCR thermal cycler must be calibrated and used according to the manufacturer's instructions.
- Do not mix components from other kits or lot numbers.
- Do not use the kit after its expiration date.
- Do not use the kit if there are suspicions of possible loss of reactivity, contamination, container deterioration or any other incidence that might affect the kits performance.
- Due to the complexity of HLA typing, data and result interpretation should be revised by qualified personnel.
- Discard expired reagents according to applicable regulations.

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Troubleshooting guide

Problem

- **Probable cause(s)**
  - Suggested corrective measure(s).

Reaction Blank (H_2O) is positive

- **Primer Mix/Master Mix/Reaction Blank contamination**
  - Repeat the experiment with new Primer Mix/Master Mix/ Reaction Blank aliquots.
  - Handle the kit components always according to accepted lab practices in order to avoid contamination.
  - Verify manipulation and storage conditions.
  - Discard contaminated reagents.
- **Pre-PCR area is contaminated**
  - Confirm that all necessary precautions in the pre-PCR area have been followed.
  - Check for possible contamination problems in other PCR techniques.
  - Confirm suitability of the used reagents (1.5 mL tubes, pipette tips).
- **Pipetting error**
  - Check that the sample added corresponds to the one indicated on the worksheet.

Low or no signal in all samples. Control samples are OK.

- **Samples with very low DNA concentration**
  - Check the DNA concentration and repeat the extraction if necessary.
- **DNA samples with high concentration**
  - Perform the assay using diluted samples.

Fluorescence intensity too low

- **Kit degradation (Primer Mix or Master Mix vials)**
  - Check that the storage of the kit is correct, reviewing both proper temperature conditions and light exposure (which should be avoided).
  - Avoid performing more than 3 thaw/freezing cycles to the reagents.

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- Aliquote the reagents if necessary.
- Repeat the test with new reagents.

Negative control sample is positive

- **Cross contamination**
  - Always handle the kit components following all necessary practices to avoid contamination.
- **Pipetting error**
  - Check that the sample added corresponds to the one indicated on the worksheet.

Positive control sample is negative

- **Pipetting error**
  - Check that the sample added corresponds to the one indicated on the worksheet.

Fluorescence intensity varies

- **The dirtiness on the outside of the tube walls interferes with the signal.**
  - Handle all consumables wearing gloves.
- **Volume is not settled to the bottom of the well or there are bubbles**
  - Perform a brief centrifugation to ensure that the volume settles to the bottom of the well and to remove all bubbles.
- **Pipetting error**
  - Check that the correct volume has been added.

There is no fluorescence signal

- **Incorrect reading channels selected**
  - Set the appropriate reading channels.
- **Pipetting error or reagent absence**
  - Control the pipetting and the reaction setup.
  - Repeat the PCR.
- **No reading channel was selected in the thermal cycler program.**
  - Check and modify the thermal cycler program.

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Changes to version 01

Version	Description of the modification
Rev.00	First document version
Rev. 01	Expression and ortographic corrections.
Rev. 02	Updating of validated qPCR thermal-cyclers.

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Explanation of symbols used on the labels

For in vitro diagnostic use

This product fulfills the requirements of Directive 98/79/EC on in vitro diagnostic medical device

Catalogue number

Lot number

Expiration date

Contents sufficient for <n> tests

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Keep away from sunlight

Positive control

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