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Bordetella bronchiseptica PCR Detection Kit — Product Insert

Product # 44900

Pathogen Information

Bordetella bronchiseptica is a gram-negative bacterium which is involved in respiratory disease in a number of different animals. In dogs, *Bordetella bronchiseptica* has been found to cause kennel cough (tracheobronchitis) and in pigs it contributes towards atrophic rhinitis. As well, this bacterium has been found to be involved in Upper Respiratory Tract Disease in cats. Feline Upper Respiratory Tract Disease may be a result of infection due to Feline Calicivirus (FCV) and Feline Herpesvirus (FHV), however *B. bronchiseptica* has been commonly isolated from cats with respiratory diseases that are FCV and FHV free. Epidemiological studies have demonstrated the bacterium is widespread in cat populations with disease being related to periods of stress, such as during pregnancy and breeding. An alarming aspect of *B. bronchiseptica* infections in cats is the amount of acute deaths which have been reported. This is especially true in young kittens when the disease can progress rapidly to bronchopneumonia. As infections can be easily treated with antibiotics, early diagnosis of the disease is crucial for the effective treatment of cats and kittens and the prevention of more serious outcomes

Principle of the Test

Norgen's *Bordetella bronchiseptica* PCR Detection Kit constituents a ready-to-use system for the isolation and detection of *Bordetella bronchiseptica* using end-point PCR. The kit first allows for the isolation of *Bordetella bronchiseptica* DNA from nasal exudates or pharyngeal swabs using spin-column chromatography based on Norgen's proprietary resin. The *Bordetella bronchiseptica* DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for *Bordetella bronchiseptica* detection using the provided *Bordetella bronchiseptica* Detection Mastermix. The *Bordetella bronchiseptica* Detection Mastermix contains reagents and enzymes for the specific amplification of a 277 bp region of the viral genome. In addition, Norgen's *Bordetella bronchiseptica* PCR Detection Kit contains a second Mastermix, the PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Digestion Buffer	10mL
Binding Solution	10mL
Wash Solution	9mL
Elution Buffer	3mL
Proteinase K	12mg
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
2x BORD Detection RT-PCR Mastermix	0.35 mL
2x PCR Control Mastermix	0.35 mL
BORD Isolation Control (IsoC)*[a]	0.3 mL
BORD Positive Control (PosC)*[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
Product Insert	1

[a] *The isolation control is a cloned DNA PCR Product.*
[b] *The positive control is a cloned B. bronchiseptica DNA PCR Product*

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- 96 – 100% ethanol
- 60°C incubator

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

Norgen's *Bordetella bronchiseptica* PCR Detection Kit contains ready-to-use Proteinase K which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K storage at 2–8°C is recommended.

The 2x BORD Detection PCR Mastermix, 2x PCR Control Mastermix, the *BORD Isolation Control (IsoC)* and the *BORD Positive Control (PosC)* should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Bordetella bronchiseptica* PCR Detection Kit, including the 2x BORD Detection PCR Mastermix, 2X PCR Control Mastermix, BORD Isolation Control and BORD Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's *Bordetella bronchiseptica* -PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Binding Solution and Wash Solution** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

General Precautions

The user should exercise the following precautions while using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

INSTRUCTIONS FOR USE

Important Notes Prior to Beginning Protocol:

- Bodily fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood or saliva.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- **BORD Isolation Control (FHV *IsoC*)**
    - A BORD Isolation Control (**BORD *IsoC***) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the BORD Isolation Control *(***BORD *IsoC****)*** to the lysate during the isolation procedure
    - The BORD Isolation Control *(***BORD *IsoC****)*** must not be added to the sample material directly.
    - Do not freeze and thaw the BORD Isolation Control *(***BORD *IsoC****)*** more than 2 times.
    - The BORD Isolation Control *(***BORD *IsoC****)*** must be kept on ice at all times during the isolation procedure.
- The PCR components of the *Bordetella bronchiseptica* Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- Acceptable specimen types include nasal exudates or pharyngeal swabs
- If using swabs, use only sterile Dacron, nylon or rayon swabs with plastic shafts. Note: Do not use calcium alginate swabs as they may contain substances that are inhibitory to PCR
- This kit is also compatible with samples collected using Norgen's Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100). Please follow the instructions provided with that kit for specimen collection and preservation.
- It is important to work quickly during this procedure.

A. Isolation of DNA

Notes:

- Ensure that all solutions are at room temperature prior to use.
- Reconstitute the Proteinase K in 0.3 mL of molecular biology grade water, aliquot in 120 µL fractions and store the unused portions at -20°C until needed.
- Add 21 mL of 96-100% ethanol to **Wash Solution**.
- Preheat a water bath or heating block to 55°C.

A. LYSATE PREPARATION

i. Nasal Exudates or Specimens previously collected using Norgen's Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100):

1. Transfer up to 150 µL of nasal exudates or preserved specimen to a 1.5 mL microcentrifuge tube (not provided). Adjust the volume to 300 µL by adding **Digestion Buffer**.
2. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

   Note: Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

3. Add 300 µL of **Binding Solution** to the lysate. Mix by vortexing
4. Add 10 µL of Isolation Control (**BORD *IsoC***) to the lysate. Vortex for 10 seconds to mix
5. Add 300 µL of 95 – 100% ethanol. Mix by vortexing.
6. Proceed to DNA Isolation (Step B).

ii. Swabs:

1. Swabs can be placed directly into an RNase-free microcentrifuge tube containing 300 µL of the **Digestion Buffer**.
2. Using sterile techniques cut the tip where the cells were collected and place into microcentrifuge tube containing the **Digestion Buffer**.
3. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

   Note: Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

4. Add 300 µL of **Binding Solution** to the lysate. Mix by vortexing
5. Add 15 µL of Isolation Control (**BORD *IsoC***) to the lysate. Vortex for 10 seconds to mix
6. Add 300 µL of 95 – 100% ethanol. Mix by vortexing.
7. Proceed to DNA Isolation (Step B).

B. SPECIMEN DNA PURIFICATION

Following the lysate preparation, DNA can be extracted from the specimens using the supplied buffers and solutions according to the following protocol:

1. Assemble a column with one of the provided collection tubes.
2. Apply the lysate with ethanol (up to 650 µL) to the column and centrifuge for 3 minutes at 5,200 *x g* (~ 8,000 RPM).

   Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed through, spin for an additional minute.

3. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Depending on lysate volume, repeat steps **B2** and **B3**.
5. Apply 500 µL of **Wash Solution** and centrifuge for one minute at 14,000 rpm.

   Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed through, spin for an additional minute.

6. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Apply 500 µL of **Wash Solution** and centrifuge for two minutes at 14,000 rpm.
8. Discard the flowthrough and reassemble the spin column with its collection tube.
9. Spin the column for 2 minutes to thoroughly dry the resin at 14,000 rpm. Discard the collection tube.
10. Place the column into a new 1.7 mL Elution tube.
11. Add 100 µL of **Elution Solution** to the column.
12. Centrifuge for 2 minutes at 2,000 rpm followed by a 2 minute spin at 14,000 rpm. Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column for an additional minute at 14,000 rpm.
13. The purified DNA sample could be used immediately for PCR as described below. It is recommended that samples be placed at -70°C for long term storage.

B. *Bordetella bronchiseptica* PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of **2X BORD Detection PCR Mastermix** and **2X PCR Control Mastermix** provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR) each.
- For each sample, one PCR reaction using the **2X BORD Detection PCR Mastermix** and one PCR reaction using **2X PCR Control Mastermix** should be set up in order to have a proper interpretation of the result.
- For every PCR run, one reaction containing BORD Positive Control (**BORD *PosC***) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of BORD Limit of Detection.

1. Prepare the PCR for sample detection (Set #1, using **2X BORD Detection PCR Mastermix**) and control detection (Set #2, using **2X PCR Control Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one BORD detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. PCR Assay Preparation

PCR Components	Volume Per PCR Reaction
2X BORD Detection PCR Mastermix Or 2X PCR Control Mastermix	**10 µL**
Sample DNA	**2.5 µL**
Nuclease-Free Water	**7.5 µL**
Total Volume	**20 µL**

2. For each PCR run, prepare **one** positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

PCR Components	Volume Per RT- PCR Reaction
2X BORD Detection PCR Mastermix Or 2X PCR Control Mastermix	**10 µL**
BORD *Positive Control (PosC)*	**10 µL**
Total Volume	**20 µL**

3. For each PCR run, prepare **one** no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
2X BORD Detection PCR Mastermix Or 2X PCR Control Mastermix	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

Therefore, at a minimum, each PCR run will contain 6 separate PCR reactions

C. PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run PCR.

Table 4. BORD Assay Program

One Step PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**5 min**
Cycle 2 (35x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

D. *Bordetella bronchiseptica* PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 15-20 µL PCR Reaction should be loaded on a 1X TAE 1.5% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided). Prepare enough agarose gel for running one set of PCR of FHV detection and one set of PCR for controls detection.
2. The PCR products should be resolved on the 1X TAE 1.7% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

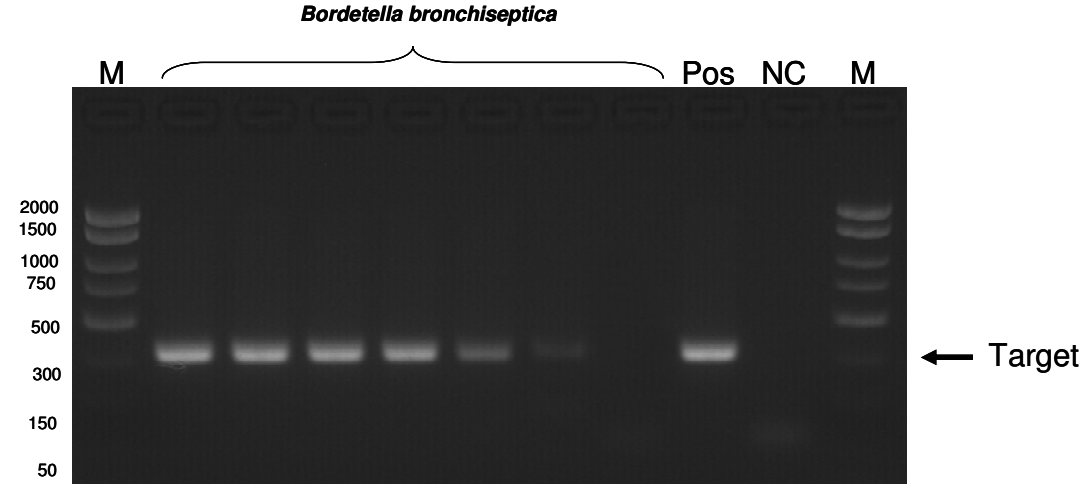

Figure 1: A representative 1.5X TAE 1.7% agarose gel showing the amplification of *Bordetella bronchiseptica* under different concentration (Target) using the 2X *Bordetella bronchiseptica* Detection PCR Mastermix. The size of the *Bordetella bronchiseptica* target amplicon corresponds to 277 bp as represented by the provided DNA Marker (M). NC = Negative Control

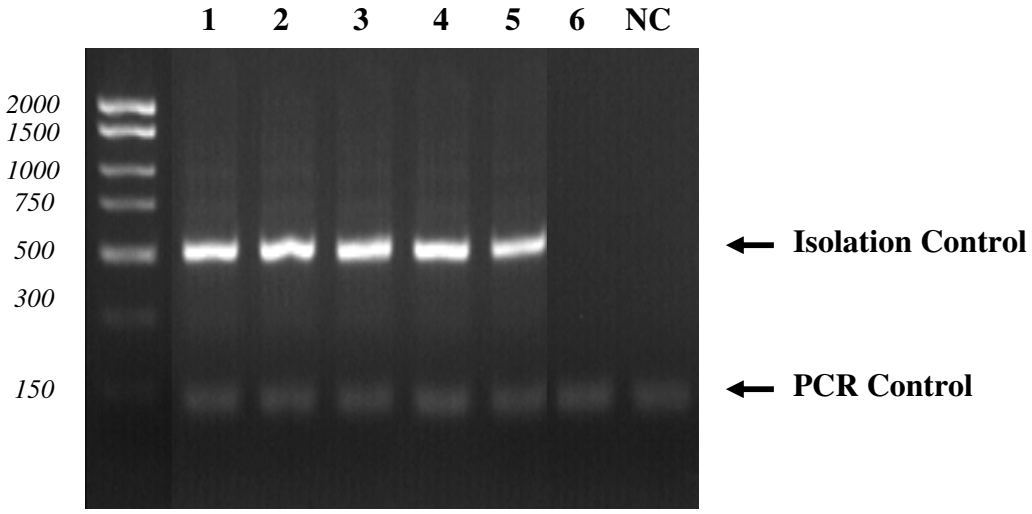

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **2X RT-PCR Control Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the RNA isolation as well as the RT-PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the RT-PCR was successful, the isolation failed to recover even the spiked-in Isolation control. **NC** = Negative Control.

Table 5. Interpretation of PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	BORD Target Band (277 bp)	**BORD *IsoC* Band (499 bp)**	**BORD *PCRC* Band (171 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

****** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. *Bordetella bronchiseptica* PCR Assay Specificity and Sensitivity

- The specificity of Norgen's *Bordetella bronchiseptica* PCR Detection Kit is first and foremost ensured by the selection of the *Bordetella bronchiseptica* specific primers, as well as the selection of stringent reaction conditions. The *Bordetella bronchiseptica* specific primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis.

F. Linear Range

- The linear range of Norgen's *Bordetella bronchiseptica* PCR Detection Kit was determined by analysing a dilution series of a *Bordetella bronchiseptica* quantification standards ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen's *Bordetella bronchiseptica* PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen's *Bordetella bronchiseptica* PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng
- Under the conditions of the Norgen's *Bordetella bronchiseptica* DNA Isolation procedure, Norgen's *Bordetella bronchiseptica* PCR Detection Kit covers a linear range from 100 copies to 1 x 10^6 copies.

Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's *Bordetella bronchiseptica* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. How can I interpret my results if neither the BORD PCR control nor the BORD Isolation Control (*IsoC*) amplifies?

- If neither the BORD PCR control nor the BORD Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the BORD PCR control showed amplification but neither the BORD target nor the BORD Isolation control amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the BORD Isolation Control (*IsoC*) was amplified in a sample?

- The sample tested can be considered as *Bordetella bronchiseptica* negative.

5. How should it be interpreted if the BORD PCR control and the BORD target showed amplification in a sample?

- The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. How should it be interpreted if only the BORD target and the BORD PCR control were amplified in a sample?

- The sample tested can be considered as *Bordetella bronchiseptica* positive.

7. How should it be interpreted if only the BORD target was amplified in a sample?

- It is recommended that the isolation is repeated.

8. How should it be interpreted if only the BORD PCR control and the BORD Isolation control showed amplification in a sample?

- The sample tested can be considered negative

9. What if I forgot to do a dry spin?

- Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the BORD Isolation Control (*IsoC*) during the isolation?

- It is recommended that the isolation is repeated.

11. What if I forgot to run the Control PCR for the sample and I only ran the Detection PCR and I obtained a positive result?

- The result can be considered positive. However, any negative result must be verified by running the associated control PCR to ensure that it is a true negative and not a false negative due to problems with the RNA isolation or the PCR reactions.

Related Products	Product #
Sample Collection Kit For Upper Respiratory Tract Infectious Agents	29100
Feline Immunodeficiency Virus RT-PCR Detection Kit	44100
Feline Calicivirus RT-PCR Detection Kit	43900
Feline Herpes Virus PCR Detection Kit	44300
Feline *infectious peritonitis* RT-PCR Detection Kit	44400

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's FeLV RT-PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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