

Quantikie™ ELISA

Human ICAM-1/CD54 Non Allele-specific Immunoassay

Catalog Number DCIM00
SCIM00
PDCIM00

For the quantitative determination of human Intercellular Adhesion Molecule 1 (ICAM-1) concentrations in cell culture supernates, serum, plasma, and urine.*

A polymorphism exists in the human ICAM-1 sequence. The antibodies used in this kit are not allele-specific. They recognize both the wild type and K29M, Kilifi mutation, ICAM-1. The Kilifi mutation variant is present in Africans at a frequency reported as high as 20% and predisposes to cerebral malaria (Fernandez-Reyes, D. et al. (1997) Hum. Mol. Genet. **6:1357).*

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Intercellular Adhesion Molecule-1 (ICAM-1), also known as CD54, is a nearly ubiquitous transmembrane glycoprotein that plays a key role in leukocyte migration and activation (1, 2). Human ICAM-1 contains five Ig-like domains in its extracellular domain (ECD) and associates into noncovalently linked dimers (3, 4). Soluble forms of monomeric and dimeric ICAM-1 can be generated via proteolytic cleavage by Cathepsin G, Elastase, MMP-9, MMP-14/MT1-MMP, and TACE/ADAM17 (5-8). Within the ECD, human ICAM-1 shares 53% amino acid sequence identity with mouse and rat ICAM-1.

The principal binding partners of ICAM-1 are the leukocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) (9-11). The multivalency of dimeric ICAM-1 increases its strength of interaction with LFA-1 (9, 10). ICAM-1 also binds several non-integrin ligands including CD43/Sialophorin, Fibrinogen, Hyaluronan, rhinoviruses, and *Plasmodium falciparum*-infected erythrocytes (12-16). At sites of inflammation, ICAM-1 is upregulated on endothelial and epithelial cells where it mediates the adhesion and paracellular migration of leukocytes expressing activated LFA-1 and Mac-1 (17-20). ICAM-1 ligation prolongs antigen presentation by dendritic cells and promotes T cell proliferation and cytokine release (21-23). ICAM-1 activation also participates in angiogenesis, wound healing, and bone metabolism (24-26).

Soluble ICAM-1 has been reported in serum, cerebrospinal fluid, urine, and bronchoalveolar lavage fluid (2, 27-31). Elevated levels of soluble ICAM-1 in these fluids are associated with cardiovascular disease, type 2 diabetes, organ transplant dysfunction, oxidant stress, increased abdominal fat mass, hypertension, liver disease, and certain malignancies (32-40). In humans, a single amino acid substitution (K29M) in ICAM-1, known as the Kilifi mutation, is associated with increased susceptibility to cerebral malaria (41). Circulating levels of ICAM-1 are elevated in cerebral malaria and correspond to the severity of disease (42, 43). This ELISA kit detects both the wild type and Kilifi varieties of soluble human ICAM-1. Soluble ICAM-1 promotes angiogenesis and serves as an indicator of vascular endothelial cell activation or damage (44, 45). It also functions as an inhibitor of transmembrane ICAM-1 mediated activities such as monocyte adhesion to activated endothelial cells and sensitivity of tumor cells to NK cell-mediated lysis (7, 8).

The Quantikine™ Human ICAM-1/CD54 Non Allele-specific Immunoassay is a 2.5 hour solid-phase ELISA designed to measure human ICAM-1 in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant human ICAM-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ICAM-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human ICAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human wild type and Kilifi variant ICAM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ICAM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human wild type and Kilifi variant ICAM-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ICAM-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DCIM00	CATALOG # SCIM00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ICAM-1 Microplate	893602	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human wild type and variant ICAM-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ICAM-1 Non Allele-specific Standard	894815	2 vials	12 vials	Recombinant human ICAM-1 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use a new standard for each assay. Discard after use.
Human ICAM-1 Non Allele-specific Conjugate	894790	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human wild type and variant ICAM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD5P	895151	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. <i>Use diluted 1:5 in this assay.</i>	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DCIM00 contains sufficient materials to run an ELISA on one 96 well plate.

SCIM00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDCIM00). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
Note: Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human ICAM-1 Microplate	893602	50 plates
Human ICAM-1 Non Allele-specific Standard	894815	50 vials
Human ICAM-1 Non Allele-specific Conjugate	894790	50 vials
Calibrator Diluent RD5P	895151	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package inserts	753017	2 booklets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-technie.com*

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 100 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human ICAM-1 Controls (optional; R&D Systems®, Catalog # QC236)

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.
Grossly hemolyzed samples are not suitable for use in this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 20 μL of sample + 380 μL of Calibrator Diluent RD5P (diluted 1:5)*.

Urine samples require a 2-fold dilution. A suggested 2-fold dilution is 150 μL of sample + 150 μL of Calibrator Diluent RD5P (diluted 1:5)*.

*Refer to the Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

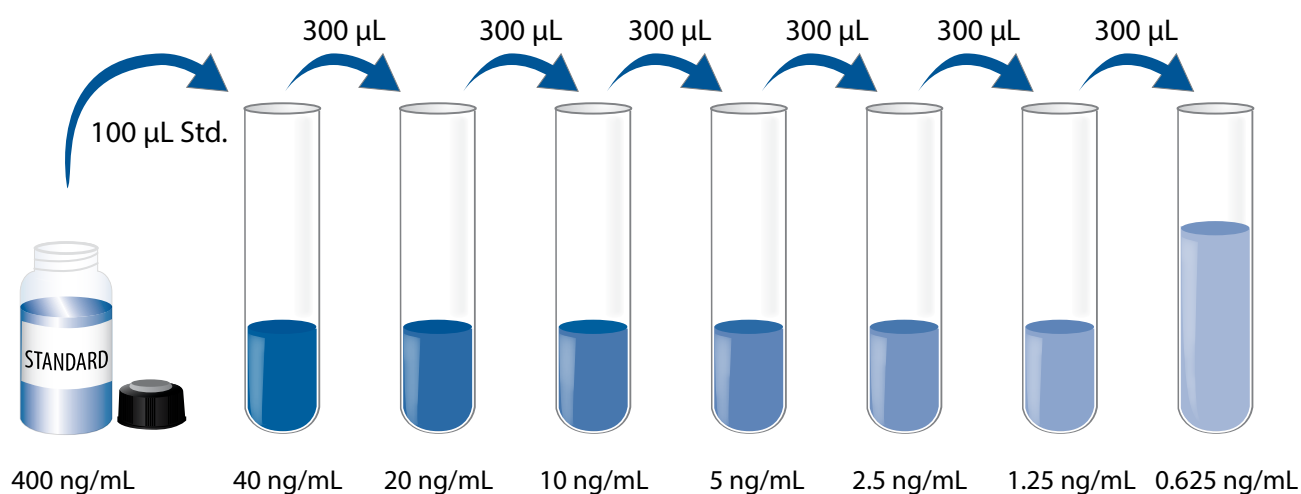
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human ICAM-1 Non Allele-specific Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human ICAM-1 Non Allele-specific Standard with deionized or distilled water. This reconstitution produces a stock solution of 400 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μ L of Calibrator Diluent RD5P (diluted 1:5) into the 40 ng/mL tube. Pipette 300 μ L into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 40 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μ L of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 200 μ L of Human ICAM-1 Non Allele-specific Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
6. Repeat the aspiration/wash as in step 4.
7. Add 200 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
8. Add 50 μ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

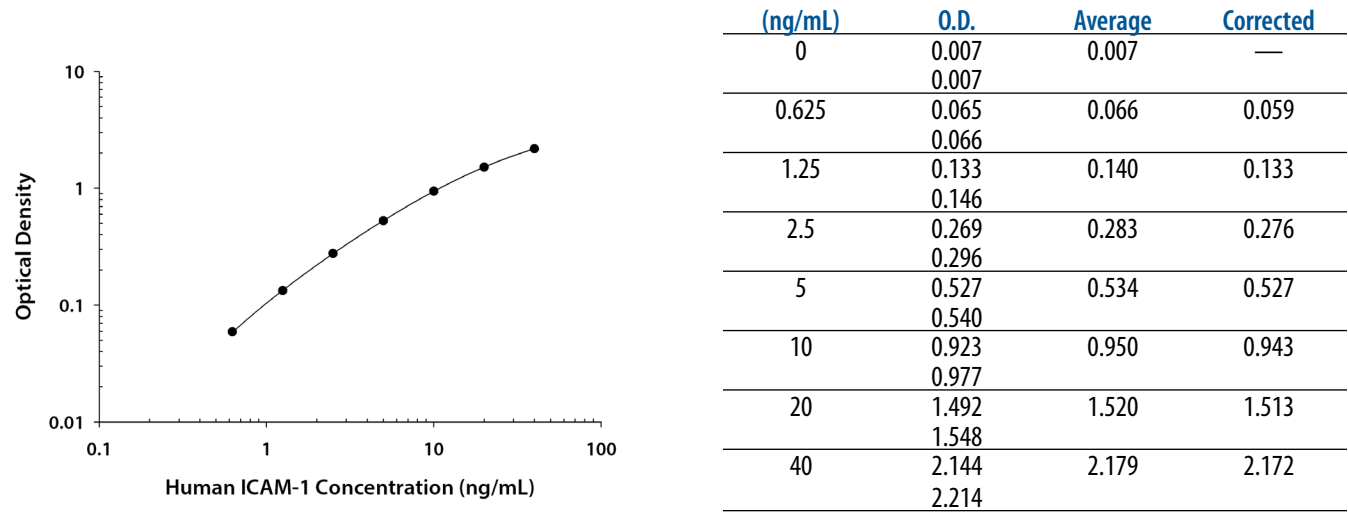
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human ICAM-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



PRECISION

Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	3.03	9.64	20.0	3.27	9.81	19.5
Standard deviation	0.128	0.346	0.973	0.282	0.543	1.22
CV (%)	4.2	3.6	4.9	8.6	5.5	6.3

RECOVERY

The recovery of human ICAM-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	96-117%
Urine* (n=4)	94	85-106%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ICAM-1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine (n=4)
1:2	Average % of Expected	100	108	107	113	108
	Range (%)	97-103	105-113	100-113	108-117	104-113
1:4	Average % of Expected	97	109	108	108	105
	Range (%)	94-101	103-116	103-110	106-110	103-108
1:8	Average % of Expected	94	112	110	107	105
	Range (%)	88-98	104-117	104-116	101-114	100-107
1:16	Average % of Expected	95	112	108	107	105
	Range (%)	92-97	104-119	99-117	103-113	101-113

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Thirty-four assays were evaluated and the minimum detectable dose (MDD) of human ICAM-1 ranged from 0.006-0.053 ng/mL. The mean MDD was 0.022 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human ICAM-1 manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human ICAM-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	306	195-859	123
EDTA plasma (n=36)	306	196-878	121
Heparin plasma (n=36)	292	172-878	124

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Urine (n=23)	3.39	91	ND-7.16

ND=Non-detectable

Cell Culture Supernates - HUVEC human umbilical vein endothelial cells were cultured in EGM™-2 Bulletkit™. Cells were cultured unstimulated or stimulated with 100 ng/mL of recombinant human TNF-α for 1 day. Aliquots of the cell culture supernates were removed, assayed for human ICAM-1, and measured 5.02 ng/mL and 25.3 ng/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant human ICAM-1.

The factors listed below were prepared at 250 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 250 ng/mL in a mid-range recombinant human ICAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ICAM-2	Integrin αM
ICAM-3	Integrin β
ICAM-4	Integrin β2
ICAM-5	
Integrin αL	

Other recombinants:

mouse ICAM-1
rat ICAM-1

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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	A	B	C	D	E	F	G	H

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