VWR® PerfectBlue™ Horizontal Mini Gel Systems Mini S, M, L & Mini L 'Revolution'

Instruction Manual

European Catalogue Numbers:

Mini S	**700-0741**
Mini M	**700-0758**
Mini L	**700-0777**
Mini L 'Revolution'	**700-0799**

Version: 1

Issued: 05,04,2018

CE

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Legal Address of Manufacturer

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Country of Origin

United States

Warranty

VWR warrants that this product will be free from defects in material and workmanship for a period of three (3) years from date of delivery. If a defect is present, VWR will, at its option and cost, repair, replace, or refund the purchase price of this product to the customer, provided it is returned during the warranty period. This warranty does not apply if the product has been damaged by accident, abuse, misuse, or misapplication, or from ordinary wear and tear. If the required maintenance and inspection services are not performed according to the manuals and any local regulations, such warranty turns invalid, except to the extent, the defect of the product is not due to such non-performance.

Items being returned must be insured by the customer against possible damage or loss. This warranty shall be limited to the aforementioned remedies. IT IS EXPRESSLY AGREED THAT THIS WARRANTY WILL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND IN LIEU OF THE WARRANTY OF MERCHANTABILITY.

Packaging List

The following items are included in shipment for the models PerfectBlue™ Mini S, Mini M, Mini L and Mini L 'Revolution':

- one buffer chamber with corrosion-protected platinum electrodes
- one safety lid with attached power cords
- one UV-transmissible gel tray with gaskets
- Mini S: 2 combs,1.5 mm thick, 6 and 10 teeth
- Mini M: 2 combs, 1.5 mm thick, 10 and 14 teeth
- Mini L ('Revolution'): 2 combs, 1.5 mm thick, 12 and 20 teeth
- User Manual

Safety Precautions

To avoid electrical shock:

- Please, read this Instruction Manual carefully before using the gel system.
- Only use a CE marked DC power supply.
- Always disconnect the gel system from the power supply before removing the safety lid.
- Always disconnect the gel system from the power supply when it is not in use or before moving it.
- Running conditions for this unit should not exceed the maximum operating voltage or current.
- Do not fill the chamber with running buffer above the maximum fill line.
- The buffer ascending tube at the lower side of the Mini L 'Revolution' gel chamber may not be used as a carry handle as it does not resist mechanical forces in perpetuity.

Intended Use

Use this product only for its intended purpose as described in this manual. Do not use this product if the power leads are damaged or if any of its surfaces are cracked.

For research use only, Not intended for use in diagnostic or therapeutic applications.

System Overview

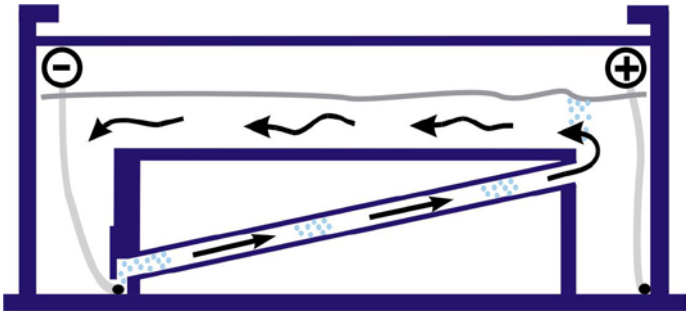
The horizontal electrophoresis systems PerfectBlue™ Mini S, M, L and Mini L 'Revolution' have been designed as 'all-in-one' systems that make it possible to cast and run gels in the same chamber. The user does not need any additional casting equipment such as grease, agarose seals or other accessories to seal the gel tray for pouring the gel.

All PerfectBlue™ horizontal Mini Gel Systems include a UV-transmissible gel tray, which has the minimum of two comb positions, allowing the user to run two sets of samples for equal distances simultaneously and a fluorescent ruler that helps in the precise photo documentation of each gel run.

In total VWR offers 6 different Mini Gel systems. In addition to the Mini S, M, L and L 'Revolution' models that are described here, two wide-format Mini Gel Systems are available (Mini ExM and Mini ExW). A comprehensive range of accessories is available for this range. These include stand-alone casting chambers for pouring up to 3 gels simultaneously while the chamber is in use, the adjustable casting chamber, a wide variety of standard combs, microtiter combs (not available for Mini S), preparative combs and wall combs that allow you to cast shorter gels in a standard gel tray. Microtiter combs allow you time-saving loading of the gels via a multi-channel pipette.

For detailed information on available accessories visit **vwr.com** or see 'Technical Support and Ordering Information'.

In contrast with all the other Mini Gel Systems, the Mini L 'Revolution' model is equipped with an internal buffer recirculation system. A trapping system captures hydrogen bubbles which are produced at the cathode due to electrolysis, and directs them through an ascending tube to the opposing side of the buffer chamber where the anode is located. During this hydrogen bubble migration, the buffer circulates, preventing the creation of detrimental pH or ion gradients.



Schematic drawing: 'Revolution'-Technology

Technical Properties

PerfectBlue™	ECN	Gel size (W x L)	Buffer volume	Voltage	Current	Time required
Mini S	700-0741	7 x 8 cm	400 ml	20 - 150 V	1 - 75 mA	30 - 60 min
Mini M	700-0758	9 x 11 cm	600 ml	20 - 150 V	1 - 75 mA	45 - 90 min
Mini L	700-0777	12 x 14 cm	800 ml	20 - 150 V	1 - 75 mA	60 - 120 min
Mini L 'Revolution'	700-0799	12 x 14 cm	1000 ml	20 - 150 V	1 - 75 mA	60 - 240 min

General Instructions

Setting Up the System and Pouring the Agarose Gel

1. Remove the lid from the gel box by holding the front of the buffer chamber with one hand and pulling the lid off by holding the center of the back of the lid.
2. For shipping and convenient storage, the gel tray is packaged inside the unit upon arrival. This is also the correct 90 ° tray position for casting a gel. To remove the gel tray, hold the unit firmly with one hand; grasp the long sides of the UVT gel tray and pull up slowly at an angle. The tray needs to fit snug for leak proof gel casting, so it may seem somewhat tight. 'Walking' the tray upwards at an angle may be helpful.
3. To cast a gel, place the gel tray into the chamber so that the gasketed ends press against the walls of the buffer chamber. Make sure the gel tray is pressed all the way down and rests level on the unit's

platform. Moistening the rubber gaskets of the gel tray may facilitate the placement into the chamber. Similarly the gel trays can be sealed in optional casting chambers.

Optional: The wall comb available for Mini L and Mini L 'Revolution' allows you to sub-divide the gel tray in order to cast shorter gels. The wall comb should be sealed with 2 % agarose before pouring the gel.

4. Use electrophoresis-grade agarose and compatible electrophoresis buffer to prepare the gel. The percentage of agarose and the buffer to be used is determined by the size of the samples to be separated and further recovery of the samples (see 'Required Reagents and Recipes'). The agarose and buffer are mixed and heated over a heat plate by stirring or in a microwave oven until the agarose is completely dissolved.
5. The prepared gel must then be cooled to below 60 °C before casting to avoid warping the UVT gel tray due to excessive heat. If numerous gels are to be run in one day, a large volume of gel may be prepared and be placed in a covered bottle stored between 40 - 60 °C in a water bath.
6. Pour or pipette the correct amount (see 'Agarose: Gel volumes and percentage') of warm agarose (< 60 °C) onto the UVT gel tray that has been placed into the casting position in the gel box. Immediately after pouring, insert the desired comb or combs into the comb slots to form the sample wells. Standard agarose should solidify completely in about 30 minutes. If low melting point or a specialty agarose is used, consult the instructions that came with the product.

Loading of Samples and Electrophoresis

1. Once the gel is completely solidified, lift the tray out of the chamber, turn it 90 °, and replace it in the chamber with the first comb closest to the cathode side (black electrode) of the chamber. The running position exposes the open ends of the agarose to the buffer.
2. Pour enough compatible running buffer into the unit to fill chamber and completely cover and submerge the gel. A 'Fill Line' is located on each unit to clearly mark the correct buffer level. See 'Technical properties' for approximate buffer volumes needed for your unit. Too little buffer may cause the gel to dry out during the run, while excess buffer may slow DNA migration in the gel, increase heat build-up and cause distorted bands.
3. Carefully remove the comb (or combs) by tapping lightly to loosen, and slowly lift straight up out of the gel tray to avoid damage to the wells.
4. Load prepared samples into the wells. Samples should be mixed with a sample loading buffer (giving weight to the samples so that they drop evenly into the wells), and contain tracking dye to monitor the gel run. For details on approximate well volumes see 'Technical Support and Ordering Information'. If Microtiter combs have been used, wells can be loaded by a multi channel pipette. Wells that have been cast with microtiter combs of 26 teeth or less can be loaded 'directly', i.e. the pipette tips fit sequentially into the gel wells.

   NOTE: It is wise to always run a sample lane of a known 'standard ladder' to determine concentration and size of separated fragments after the gel run, and to aid in photo documentation and analysis.
5. Carefully slide the lid with attached power cords onto the unit. This will connect the power cords to the banana plugs to complete the circuit. Plug the other end of the cords (4 mm, male) into an appropriate power supply; take care to the proper orientation of the electrical field. Remember that nucleic acids are negatively charged in an alkaline to neutral surrounding and therefore will migrate to the positively charged anode. In general, the color coding for positively charged electrodes is red.
6. Turn on the power supply and run the gel at the appropriate voltage/current (see 'Technical properties'). You can observe the progress of electrophoresis by the visual migration of the loading dye. Note that in 0.5 x TBE gels bromophenol blue co-migrates at 300 bp and xylene cyanol at 4 kbp with the DNA fragments.

Visualization

When the tracking dye has migrated as far through the gel as desired, or to the end of the gel, turn off the power supply and slide off the lid to disconnect from the power source. Carefully remove the tray containing the gel (wear gloves). The UV-transmissible gel tray makes for simple visualization and photography with a UV light source without the need to remove the gel from the tray. The gel tray may be placed back into the casting chamber for convenient transport to the darkroom and to avoid damage to the gel.

Cleaning

The buffer chamber and tray should be rinsed under warm running water after each use. Use a mild detergent to get rid of any debris. A following short rinse off with distilled water prevents the formation of salt marks. It is recommended to allow the chamber to air dry rather than drying with a towel to avoid damage to the electrode wires. Do not use ethanol or other organic solvents to clean acrylic products, because organic solvents cause acrylic to 'craze' or crack!

Required Reagents and Recipes

Electrophoresis Buffers

Electrophoresis buffers supply the ions necessary for electrophoresis and establishing a certain pH value in which the target molecule adapts to the required electric charge. Nucleic acids will be negatively charged in an alkaline to neutral surrounding. Additionally, electrophoresis buffers often contain reagents which protect the target molecule from degradation (e.g. EDTA, which complexes bivalent cations and therefore inhibits DNases). If electrophoresis under denaturing conditions is desired (like for the electrophoresis of RNA), electrophoresis buffers will additionally contain reagents that eliminate the formation of secondary structures. You will find recipes below for TAE and TBE, two of the most commonly used buffers for the electrophoresis of DNA. If the intention is to eventually isolate DNA from the gel, TAE buffer should be chosen. In comparison to TBE, migration will be faster and a better resolution of supercoiled DNA will be achieved when using TAE. However, because of TAE's limited buffering capacity, TBE should be selected for performing extended electrophoresis separations and if the electrophoresis chamber does not possess a system for buffer recirculation. VWR's PerfectBlue 'Revolution' Systems are equipped with an internal buffer recirculation system that prevents the formation of pH and ion gradients during extended runs. Since agarose tends to create finer pore sizes and a more solid matrix in TBE, diffusion of DNA will be reduced and a more discrete band pattern will be achieved.

TAE (Tris-Acetate-EDTA) Buffer

1 x working solution:	40 mM Tris-acetate, 1 mM EDTA
50 x stock solution (1 L):	242 g Tris-Base
	57.1 ml Glacial acetic acid
	100 ml 0.5 M EDTA (pH 8.0)
	Adjust volume to 1L using distilled H2O

TBE (Tris-Borate-EDTA) Buffer

0.5 x working solution*: 45 mM Tris-Borate, 1 mM EDTA

5 x stock solution (1 L)**:	54 g Tris-Base
	27.5 g Boric acid
	20 ml 0.5 M EDTA (pH 8.0)
	Adjust volume to 1L using distilled H2O

* 0.5 x TBE is sufficient for agarose gel electrophoresis. For vertical electrophoresis in polyacrylamide gels, 1 x TBE is often applied due to the comparatively smaller buffer reservoirs of vertical electrophoresis chambers.

** 5 x TBE stock solutions tend to precipitate during long storage periods and should get remade. Because of this property, higher concentrations of TBE stock solutions should be avoided.

Agarose: Gel Volumes and Percentage

VWR offers an extensive range of high quality agaroses, for many specific applications (see 'Technical support and ordering information').

The required volume of the gel is calculated using the following formula.

gel width (cm) x gel length (cm) x gel thickness (cm) = required volume agarose solution (ml)

The following volumes will result:

Model	Gel size (cm)	Gel thickness (cm)			
		0.25	0.5	0.75	1.0
PerfectBlue Mini S	7 x 8 (W x L)	14 ml	28 ml	42 ml	56 ml
PerfectBlue Mini M	9 x 11 (W x L)	25 ml	50 ml	75 ml	100 ml
PerfectBlue Mini L ('Revolution')	12 x 14 (W x L)	42 ml	84 ml	126 ml	168 ml

The optimal range of DNA fragment sizes separated by any electrophoresis experiment is dependent on the agarose concentration of the gel. The higher the agarose concentration, the better small fragments are separated from each other and vice versa. However, for the smallest or largest fragment lengths, the usage of specialized agaroses or polyacrylamide gels should be considered (see table below) since a 3 % agarose solution solidifies rapidly and a 0.3 % agarose gel is very soft and difficult to handle.

Agarose content (w/v)	Agarose (g)	Buffer (ml)	optimal separation range (kb)
0.3 %	0.3	100	5 - 30
0.5 %	0.5	100	1 - 15
0.7 %	0.7	100	0.8 - 10
1.0 %	1.0	100	0.5 - 7
1.2 %	1.2	100	0.3 - 6
1.5 %	1.5	100	0.2 - 4
2.0 %	2.0	100	0.1 - 3
3.0 %	3.0	100	< 0.1

Ethidium Bromide

The gel may be stained during or following the run with a variety of stains for photo documentation. The most common stain for DNA is ethidium bromide. Because of its capacity to intercalate into double stranded nucleic acids and alter the conformation of DNA, ethidium bromide is judged to be highly mutagenic. Therefore appropriate safety measures must be applied.

Ethidium bromide may be added directly to the gel before pouring it at a concentration of 0.1 to 0.5 µg/ml. However, being positively charged, ethidium bromide will migrate to the cathode during the electrophoresis leading to uneven staining. Improved results can be obtained by incubating the gel after the electrophoresis is finished in electrophoresis buffer containing 0.5 µg/ml ethidium bromide for 5 to 20 min. Subsequently the gel should get rinsed in electrophoresis buffer without ethidium bromide for up to 20 min in order to reduce background signal.

Loading Buffer/Sample Buffer

Samples are prepared and mixed with loading buffer before applying to the prepared gel. Sample buffers contain dyes for visibility and glycerol to provide weight to the samples. This increased sample density ensures samples load evenly into the wells and do not float out during loading. Dyes also migrate toward the anode end of the electrophoresis chamber at predictable rates allowing the gel run to be monitored. In 0.5 x TBE gels, bromophenol blue migrates at the same rate as 300 bp DNA fragments and xylene cyanol approximately at the same rate as 4 kbp DNA fragments.

6 x DNA sample buffer: 0.25 % (w/v) bromophenol blue
0.25 % (w/v) xylene cyanol FF
30 % (v/v) glycerol

Molecular Weight Marker

Markers are run on each gel to monitor the quality of sample separation and to enable a size estimation of specific bands. By running a known marker of a specific concentration in parallel, the DNA amount of the unknown samples can be estimated. VWR offers an extensive range of DNA and RNA markers. For detailed information please contact us or visit **vwr.com**.

Troubleshooting

Some possible solutions to potential problems are listed below. If these suggestions are unclear or unsuccessful, please contact your local VWR-Service-Team.

Problem: Agarose leaks into chamber when pouring gel

Check to see if the gasket is firmly seated in the grooves on the ends of the UVT gel tray. Reseat gasket if necessary by removing and rinsing under warm running water, then reseat evenly in the tray groove.

Problem: Bands appear diffused in the gel or bands seem to be running at an angle (Gel smiling).

Check to be sure the casting is being done on a level surface. Also confirm that the gel tray is inserted all the way into the unit and rests on the platform for level gel casting. The voltage may be too high. Try lowering the voltage setting on the power supply. Electrophoresis buffer may have been prepared with wrong or expired chemicals. Perhaps no electrophoresis buffer has been used for preparing the gel solution. Check if the stock solution of the electrophoresis buffer was diluted correctly for the preparation of the working solution.

Problem: Samples seem to be running unevenly in certain areas.

Check that the platinum electrode wire is intact and running evenly across the base of the chamber and up the side to the junction of the banana plug. The unit should produce small bubbles as the current passes through. If there appears to be a break in the electrode connection, contact VWR immediately. This problem may also be caused by regularly casting with very hot agarose gel (> 60 °C). Always cool the melted agarose to below 60 °C before casting to avoid warping the UVT gel tray. Warping the gel tray will cause all subsequent gels to be cast unevenly and hence cause bad electrophoretic separation.

Problem: Samples do not band sharply.

Gels should be allowed to solidify completely before running. Standard agarose should solidify in about 30 minutes. If low melting point agarose is used, it may be necessary to completely solidify gels at a cooler temperature in the refrigerator or cold room. Gels should be submerged in 3 - 5 mm of buffer to prevent the gel from drying out, but excess buffer (> 5 mm) can cause decreased DNA mobility and band distortion. Perhaps too much nucleic acid was applied to the wells potentially leading to 'smearing' of the bands.

Problem: Samples are remaining in the wells, running 'backwards' or diffusing out of the gel.

Check that a complete power circuit is achieved between the unit and the power supply. Platinum wire and banana plugs should be intact. To test, simply fill the unit with running buffer and attach to the power supply without a gel or gel tray in the unit. The platinum wires on both sides of the unit should produce small bubbles as the current passes through. If a complete circuit does not exist there will be little to no bubbles. If

samples appear to run backwards through the gel or there are no bands visible, check to be sure that the gel tray was placed in the electrophoresis chamber in the proper orientation. If the orientation or polarity is reversed, the samples will run backwards or migrate off the gel. The tray should be placed in the chamber with the comb at the edge of the tray closest to the cathode side (black) of the chamber.

Problem: When the comb is removed from the gel the sample well is ripped and damaged.

Always make sure to allow the gel to solidify completely and note that the gel should be submerged by running buffer before moving the tray, unit, or removing the comb. To avoid damage to the sample wells, gently rock the comb back and forth lightly to loosen and then slowly pull the comb straight up out of the gel tray. This rocking helps to avoid suction as the comb is removed.

Problem: The gel seems to run slower under the usual running conditions.

The volume of running buffer used to submerge the gel should only be between 3 - 5 mm over the gel surface. Gel should be completely submerged to avoid the gel from drying out and to assure that a uniform electrical field can be generated. However, if excessive running buffer is used, the current flow through the gel and the migration of the DNA is decreased.

Technical Support and Ordering Information

For technical questions and more detailed information on VWR's products please contact your local VWR office or visit the VWR website at **vwr.com**.

Visit the VWR website at **vwr.com** for:

- Complete technical service contact information
- Access to the VWR Online Catalogue, and information about accessories and related products
- Additional product information and special offers

PerfectBlue™ Mini S

Item	Description			European Cat. No.
Gel system Mini S	complete system for gels 7 x 8 cm (W x L)			700-0741
Casting chamber	Casting chamber for up to 3 gel trays			700-0753
Gel tray	UV-transmissible gel tray and gaskets			700-0757
Gaskets	2 rubber gaskets for gel tray			700-0754
Standard combs	1.5 mm	5 teeth	64 µl*	700-0747
	1.5 mm	6 teeth	51 µl*	700-0749
	1.5 mm	8 teeth	36 µl*	700-0751
	1.5 mm	10 teeth	26 µl*	700-0743
	1.5 mm	12 teeth	21 µl*	700-0745
	1.0 mm	5 teeth	42 µl*	700-0746
	1.0 mm	6 teeth	34 µl*	700-0748
	1.0 mm	8 teeth	24 µl*	700-0750
	1.0 mm	10 teeth	18 µl*	700-0742
	1.0 mm	12 teeth	14 µl*	700-0744
Preparative comb	1.5 mm	2 teeth	320/28 µl*	700-0756

* volumes are calculated for a gel thickness of 5 mm

PerfectBlue™ Mini M

Item	Description	European Cat. No.
Gel system Mini M	complete system for gels 9 x 11 cm (W x L)	700-0758
Casting chamber	Casting chamber for up to 3 gel trays	700-0773

Gel tray	**UV-transmissible gel tray and gaskets for up to 4 combs**			**700-0776**
Gaskets	2 rubber gaskets for gel tray			700-0774
Standard combs	1.5 mm	5 teeth	86 µl*	700-0768
	1.5 mm	8 teeth	51 µl*	700-0770
	1.5 mm	10 teeth	38 µl*	700-0760
	1.5 mm	12 teeth	30 µl*	700-0762
	1.5 mm	14 teeth	25 µl*	700-0764
	1.0 mm	5 teeth	58 µl*	700-0767
	1.0 mm	8 teeth	34 µl*	700-0769
	1.0 mm	10 teeth	25 µl*	700-0759
	1.0 mm	12 teeth	20 µl*	700-0761
	1.0 mm	14 teeth	16 µl*	700-0763
Microtiter combs	1.5 mm	9 teeth	40 µl*	700-0772
	1.5 mm	18 teeth	16 µl*	700-0766
	1.0 mm	9 teeth	27 µl*	700-0771
	1.0 mm	18 teeth	11 µl*	700-0765
Preparative comb	1.5 mm	2 teeth	439/28 µl*	700-0775

* volumes are calculated for a gel thickness of 5 mm

PerfectBlue™ Mini L & Mini L 'Revolution'

The same accessories are used for both models, Mini L and Mini L 'Revolution'.

Item	Description			European Cat. No.
Gel system Mini L	complete system for gels 12 x 14 cm (W x L)			700-0777
Gel system Mini L 'Revolution'	complete system for gels 12 x 14 cm (W x L)			700-0799
Casing chamber	Casting chamber for up to 3 gel trays			700-0792
Gel tray L4	UV-transmissible gel tray and gaskets, for up to 4 combs			700-0797
Gel tray L12	UV-transmissible gel tray and gaskets, for up to 12 combs			700-0796
Gaskets	2 rubber gaskets for gel tray			700-0793
Wall comb	Wall comb for dividing up the gel tray			700-0798
Standard combs	1.5 mm	8 teeth	70 µl*	700-0789
	1.5 mm	16 teeth	30 µl*	700-0781
	1.5 mm	20 teeth	22 µl*	700-0783
	1.5 mm	24 teeth	17 µl*	700-0785
	1.0 mm	8 teeth	47 µl*	700-0788
	1.0 mm	16 teeth	20 µl*	700-0780
	1.0 mm	20 teeth	15 µl*	700-0782
	1.0 mm	24 teeth	11 µl*	700-0784
Microtiter combs	1.5 mm	9 teeth	40 µl*	700-0791
	1.5 mm	12 teeth	40 µl*	700-0779
	1.5 mm	25 teeth	16 µl*	700-0787
	1.0 mm	9 teeth	27 µl*	700-0790

	1.0 mm	12 teeth	27 µl*	700-0778
	1.0 mm	25 teeth	11 µl*	700-0786
Preparative comb	1.5 mm	2 teeth	596/28 µl*	700-0795

* Volumes are calculated for a gel thickness of 5 mm

Adjustable Casting Chamber

For the simple, leak-proof casting of up to three Mini S gels, two Mini M gels, two Mini L gels, one Mini ExM gel or one Mini ExW gel.

Item	Description	European Cat. No.
Adjustable Caster	Adjustable Casting Chamber for PerfectBlue™ Mini Gel Systems, including a 3-point leveling system with water level	700-0849

Power Supplies

Do not hesitate to contact us for advice on which Power Supply is most suitable for your application.

Literature

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BOOTS S. (1989) Gel Electrophoresis of DNA. Anal. Chem., 61 (8): 551a-553a.

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