Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric)

Base Catalog # P-3104

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric) is designed to rapidly examine the selectivity and specificity of antibodies against 46 different histone modifications and is also suitable for identifying substrates of histone modifying enzymes and analyzing specificity of histone binding proteins.

Input Material: For antibody screening, the input materials are various IgG antibodies of interest. For histone modifying enzyme/protein screening, purified enzymes/proteins should be used. The amounts or concentrations of input materials are dependent on the assay types and can be determined by the users.

Internal Control: A negative control (non-peptide) and a universal positive control (protein A/G) are provided in this kit. The negative control can be used to check whether the background signals are properly removed, whereas the universal positive control is suitable for antibodies raised from any species and determines whether the antibody binds to the peptide properly.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	96 Assays Cat. #P-3104-96	Storage Upon Receipt
WB (10X Wash Buffer)	28 ml	4°C
DS (Developer Solution)	12 ml	4°C
SS (Stop Solution)	10 ml	RT
Array Plate (96-well)	1	4°C
Adhesive Covering Film	1	RT

SHIPPING & STORAGE

The kit is shipped at ambient room temperature. Upon receipt: (1) store **WB**, **DS**, and **Array Plate** at 4°C away from light; and (2) store **SS** and **Adhesive Covering Film** at room temperature away from light. All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: *(1) Check if **WB** (10X Wash Buffer) contains salt precipitates before use. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; and (2) check if a blue color is present in **DS** (Developer Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Distilled water
- ☐ Primary antibodies, proteins and/or enzymes of interest
- ☐ Secondary antibody conjugated with HRP
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

Histone modifications have also been defined as epigenetic modifiers. Post-translational modifications of histones include the acetylation on specific lysine residues by histone acetyltransferases (HATs), the deacetylation by histone deacetylases (HDACs), the methylation of lysine and arginine residues by histone methytransferases (HMTs), the demethylation of lysine residues by histone demethylases (HDMTs), and the phosphorylation of specific serine groups by histone kinases (HKs). Next to DNA methylation, histone acetylation and histone methylation are the most well-characterized epigenetic marks. Generally, tri-methylation at H3-K4, H3-K36, or H3-K79 results in an open chromatin configuration and is, therefore, characteristic of euchromatin. Euchromatin is also characterized by a high level of histone acetylation, which is mediated by histone acetyltransferases. Conversely, histone deacetylases have the ability to remove this epigenetic mark, which leads to transcriptional repression. Lysine residues can be mono-, di-, or tri-methylated, each of which can differentially regulate chromatin structure and transcription. Along with other histone modifications such as phosphorylation, this enormous variation leads to a myriad of possible combinations of different modifications. This might constitute a “histone code”, which can be read and interpreted by different cellular factors.

In detection of various histone modifications, especially closely related ones, it is critical that an antibody directed against modified histone H3 is highly specific with detection of only one specific type of modification and can discriminate between similarly modified histones. To screen and verify such an antibody, an adequate modified histone H3 array platform is required. Conventional histone peptide array methods have some disadvantages: (a) time-consuming; (b) require highly specialized equipment; and, in particular (c) have an insufficient variety of modified histones in the array. To address these shortcomings, EpigenTek provides the Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric) with the following advantages:

- Quick and efficient procedure, which can be finished within 1 hour and 45 minutes.
- High quality peptides (over 95% pure) are purified by HPLC and verified with mass spectrometry, and then coated in two concentrations (2 ng and 20 ng), which allows for the screening of both strong and weak reactive antibodies, enzymes, and histone binding proteins.
- The most comprehensive variety of histone H3 modifications, which contain 46 single modifications including the most important methylation (36), acetylation (8) and phosphorylation (2) modifications.
- Detection in ELISA format, which makes the assay easy and convenient.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric) is designed to rapidly examine the selectivity and specificity of antibodies against 46 different histone modifications and is also suitable for identifying substrates of histone modifying enzymes as well as for analyzing specificity of histone binding proteins. In an assay with this kit, the histone H3 proteins modified at specific sites that are tightly arrayed on the wells through biotin-avidin binding are incubated with input materials such as antibodies, proteins or enzymes. After incubation, the specifically bound input materials are detected through an ELISA reaction system (antibody-signal development reagent). The binding intensity of input materials is proportional to the intensity of absorbance.

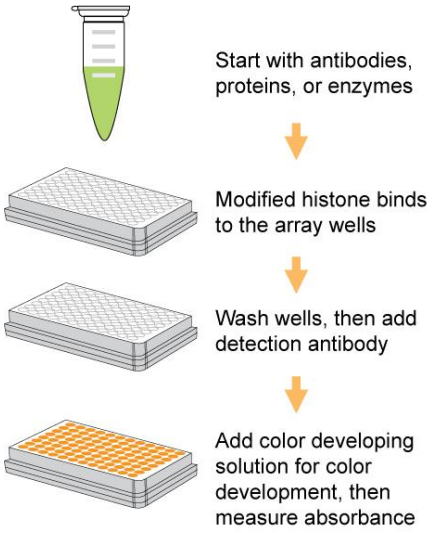


Fig 1. Schematic procedure of the Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric)

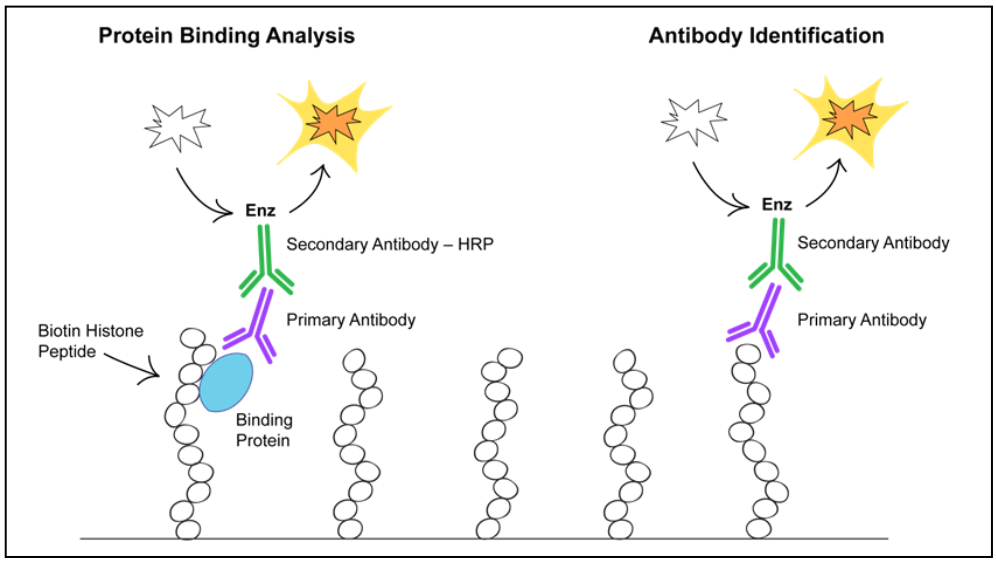
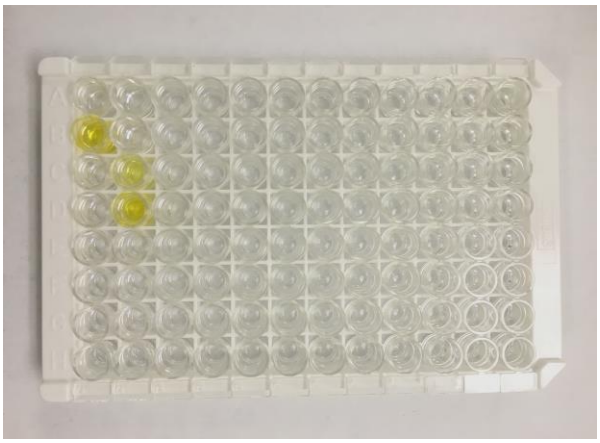


Fig 2. Working principle of Pre-Sure™ Histone H3 Peptide Array ELISA Kit (Colorimetric)

A



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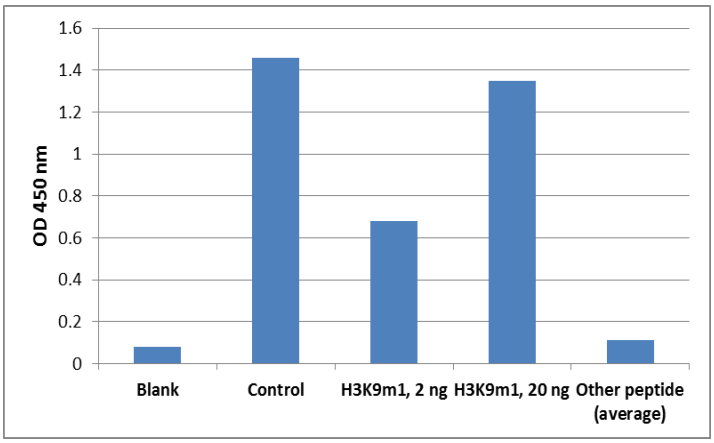

Fig 3. Representative specificity identification of histone H3 antibodies. The Histone H3 Array was probed with H3K9me1 Polyclonal Antibody (Cat. #A-4034; 1 µg/mL). Peptides and control were visualized using a goat anti-rabbit IgG-HRP and a color development system**. A**: original image; **B**: graphical analysis of specificity and binding intensity of H3K9me1 antibody.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Sample Antibodies: The sample antibodies should be diluted with 1X wash buffer with final concentration of 1 µg/ml. (See Step 1b of Protocol)

Sample Proteins: Prepare 5 ml of sample protein solution by diluting the sample protein with appropriate protein binding buffer to the desired concentration. Make sure the necessary co-factors are included in the buffer. Use the appropriate primary antibody and species-specific secondary antibody accordingly. (See Step 1b and 1c of Protocol for the dilution of antibodies)

Sample Enzymes: Prepare 5 ml of sample enzyme solution by diluting the enzyme with appropriate enzyme assay buffer to the desired concentration. Make sure the co-factors such as methyl donor-Adomet (for histone methyltransferase) are included in the assay buffer. Use the appropriate primary antibody and species-specific secondary antibody accordingly. (See Step 1b and 1c of Protocol for the dilution of antibodies)

Note: *Keep each of the diluted sample solutions on ice until use. Any remaining diluted solutions should be discarded if not used within the same day.*

1. Buffer and Solution Preparation

a. Prepare **Diluted WB** 1X Wash Buffer:

96-Assays: Add 26 ml of **WB** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **DPA** (Diluted Primary Antibody) Solution:

Dilute **Primary Antibody** with **Diluted WB** 1X Wash Buffer to 1 µg/ml (i.e., add 1 µl of primary antibody at the concentration of 1 mg/ml to 1000 µl of **Diluted WB**). 50 µl of **DPA** will be required for each assay well.

c. Prepare **DSA** (Diluted Secondary Antibodies conjugated with HRP) Solution:

Dilute **Secondary Antibody** with **Diluted WB** 1X Wash Buffer to 0.4 µg/ml (i.e., add 1 µl of primary antibody at concentration of 1 mg/ml to 2500 µl of **Diluted WB**). 50 µl of **Diluted Primary Antibody** will be required for each assay well. The secondary antibody should be specific against the primary antibody.

Note: *Keep each of the diluted solutions except* ***Diluted WB*** *1X Wash Buffer on ice until use. Any remaining diluted solutions other than* ***Diluted WB*** *should be discarded if not used within the same day.*

2. For Antibody Analysis

a. Add 50 µl of **DPA** prepared at Step 1b to each well of **Array Plate**.

b. Tightly cover the plate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60 min.

c. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

d. Add 50 µl of the **DSA** prepared at Step 1c to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min.

e. Remove the **DSA** solution from each well.

f. Wash each well four times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

Note: *Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.*

g. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue if the sample antibody binds to the histone peptides.

h. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs. (2) If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

3. For Histone Binding of Protein

a. Add 50 µl of diluted sample protein solution into each well of **Array Plate**.

b. Tightly cover the plate with **Adhesive Covering Film** to avoid evaporation and incubate at an appropriate temperature for desired time.

c. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

d. Add 50 µl of **DPA** prepared at Step 1b to each well of **Array Plate**.

Note: *The primary antibody should be specific for the sample proteins.*

e. Tightly cover the plate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60 min.

f. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

g. Add 50 µl of the **DSA** prepared at Step 1c to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min. The secondary antibody should be specific against the primary antibody used at Step 3d.

h. Remove the **DSA** solution from each well.

i. Wash each well four times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

Note: *Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.*

j. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient histone-sample protein binding.

k. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

4. For Enzyme Interaction

a. Add 50 µl of diluted sample enzyme solution into each well of **Array Plate**.

b. Tightly cover the plate with **Adhesive Covering Film** to avoid evaporation and incubate at an appropriate temperature for desired time.

c. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

d. Add 50 µl of **DPA** prepared at Step 1b to each well of **Array Plate**.

Note: *The primary antibody should be specific for the sample enzymes.*

e. Tightly cover the plate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60 min.

f. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

g. Add 50 µl of the **DSA** prepared at Step 1c to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min. The secondary antibody should be specific against the primary antibody used at Step 3d.

h. Remove the **DSA** solution from each well.

i. Wash each well four times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

Note: *Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.*

j. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient enzyme-substrate peptide binding.

k. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

5. Data Analysis

Calculate the Binding Intensity of samples using the following formula:

Binding Intensity = Sample OD - Blank OD

HISTONE PEPTIDE ARRAY MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank**	**K4m3** 2 ng	**K14m1** 2 ng	**K18m2** 2 ng	**K23m3** 2 ng	**K36m1** 2 ng	**K79m2** 2 ng	**K14ac** 2 ng	**K56ac** 2 ng	**H3R2m2s** 2 ng	**H3R17m1** 2 ng	**H3R26m2a** 2 ng
B	**Protein A/G**	**K4m3** 20 ng	**K14m1** 20 ng	**K18m2** 20 ng	**K23m3** 20 ng	**K36m1** 20 ng	**K79m2** 20 ng	**K14ac** 20 ng	**K56ac** 20 ng	**H3R2m2s** 20 ng	**H3R17m1** 20 ng	**H3R26m2a** 20 ng
C	**H3 protein**	**K9m1** 2 ng	**K14m2** 2 ng	**K18m3** 2 ng	**K27m1** 2 ng	**K36m2** 2 ng	**K79m3** 2 ng	**K18ac** 2 ng	**K79ac** 2 ng	**H3R8m1** 2 ng	**H3R17m2a** 2 ng	**H3R26m2s** 2 ng
D	**H3 protein**	**K9m1** 20 ng	**K14m2** 20 ng	**K18m3** 20 ng	**K27m1** 20 ng	**K36m2** 20 ng	**K79m3** 20 ng	**K18ac** 20 ng	**K79ac** 20 ng	**H3R8m1** 20 ng	**H3R17m2a** 20 ng	**H3R26m2s** 20 ng
E	**K4m1** 2 ng	**K9m2** 2 ng	**K14m3** 2 ng	**K23m1** 2 ng	**K27m2** 2 ng	**K36m3** 2 ng	**K4ac** 2 ng	**K27ac** 2 ng	**H3R2m1** 2 ng	**H3R8m2a** 2 ng	**H3R17m2s** 2 ng	**H3S10p** 2 ng
F	**K4m1** 20 ng	**K9m2** 20 ng	**K14m3** 20 ng	**K23m1** 20 ng	**K27m2** 20 ng	**K36m3** 20 ng	**K4ac** 20 ng	**K27ac** 20 ng	**H3R2m1** 20 ng	**H3R8m2a** 20 ng	**H3R17m2s** 20 ng	**H3S10p** 20 ng
G	**K4m2** 2 ng	**K9m3** 2 ng	**K18m1** 2 ng	**K23m2** 2 ng	**K27m3** 2 ng	**K79m1** 2 ng	**K9ac** 2 ng	**K36ac** 2 ng	**H3R2m2a** 2 ng	**H3R8m2s** 2 ng	**H3R26m1** 2 ng	**H3S28p** 2 ng
H	**K4m2** 20 ng	**K9m3** 20 ng	**K18m1** 20 ng	**K23m2** 20 ng	**K27m3** 20 ng	**K79m1** 20 ng	**K9ac** 20 ng	**K36ac** 20 ng	**H3R2m2a** 20 ng	**H3R8m2s** 20 ng	**H3R26m1** 20 ng	**H3S28p** 20 ng

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the correct amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use.
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Antibody concentration is too high.	Ensure the antibodies are appropriately diluted.
	Incubation time with **DSA** is too long.	The incubation time with **DSA** should not exceed 60 min.
	Over-development of color.	Decrease the development time with **DS** (Developer Solution) before adding **SS** (Stop Solution).
No signal or weak signal only in sample wells	Antibody concentration is too low.	Ensure the antibodies are appropriately diluted.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of sample antibodies or proteins or enzymes is added.
	The primary antibody may have lost activity due to multiple freeze-thaw cycles, or bacterial contamination has changed the activity of an antibody.	Use new antibodies and avoid cross-contamination from other assays.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residual washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure **DS** (Developer Solution) or **SS** (Stop Solution) is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

RELATED PRODUCTS

Protein Extract Preparation

OP-0002 EpiQuik™ Nuclear Extraction Kit

ChIP-Grade Antibody Validation

P-2031 Pre-Sure™ ChIP Antibody Validation Kit

Histone Modification Screening

P-3100 EpiQuik™ Histone H3 Modification Multiplex Assay Kit (Colorimetric)
P-3102 EpiQuik™ Histone H4 Modification Multiplex Assay Kit (Colorimetric)
P-3106 EpiQuik™ Histone H4/H2A/H2B Peptide Array ELISA Kit (Colorimetric)

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