

ARBOR ASSAYS™
Interactive Assay Solutions™



DetectX[®]

**DEHYDROEPIANDROSTERONE SULFATE
(DHEA-S)
Enzyme Immunoassay Kit**

1 Plate Kit Catalog Number K054-H1

5 Plate Kit Catalog Number K054-H5

Species Independent

Sample Types Validated:

**Serum, Plasma, Urine, Saliva
Dried Fecal Extracts, and
Tissue Culture Media**

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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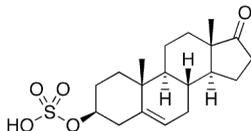
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BACKGROUND

Dehydroepiandrosterone sulfate, $C_{19}H_{28}O_5S$, (5-androsten-3 β , 16 α -diol-17-one sulfate, DHEA-S) is the major C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. It is produced by the addition of a sulfate group to dehydroepiandrosterone (DHEA), catalyzed by the sulfotransferase enzymes, SULT1A1 and SULT1E1, which also produce estrone sulfate from estrone. DHEA sulfate can also be back-converted to DHEA through the action of steroid sulfatase. DHEA-S has relatively low androgenic activity due to the 17-ketone group rather than hydroxyl group¹. However the bioactivity of DHEA-S may be high due to its high serum concentrations at 100-1,000-fold higher than testosterone or DHEA and its weak affinity for sex-hormone binding globulin².



Dehydroepiandrosterone sulfate

The physiological role of DHEA-S is not well defined, with serum levels being high in the fetus and neonates, low during childhood and increased during puberty^{3, 4}. DHEA-S levels decline during the third decade of life⁵. DHEA-S, unlike DHEA and other steroids, does not show a significant diurnal or day-to-day variation. DHEA-S levels are not increased due to ACTH administration and do not change significantly during the normal menstrual cycle^{2, 4}. DHEA-S has a lower metabolic clearance rate than DHEA⁶.

Since DHEA-S is primarily produced by the adrenal glands, it is useful as a marker for adrenal function. Adrenal tumors, cancers, and hyperplasia can lead to the overproduction of DHEA-S. While elevated levels may not be noticed in adult men, they can lead to amenorrhea and visible symptoms of virilization. These changes vary in severity and may include a deeper voice, hirsutism, male pattern baldness, muscularity, acne and enlargement of the Adam's apple. Women with polycystic ovary syndrome tend to have elevated levels of DHEA-S. Excess levels of DHEA-S in children can cause precocious puberty in boys; and ambiguous external genitalia, excess body hair, and abnormal menstrual periods in girls.

1. Dorfman, RI and Shiple, RA., *Androgens*, J. Wiley and Sons, NY, 1956, 116-128.
2. Pang, S. and Riddick, L., "Pediatric Endocrinology, A Clinical Guide, 2nd Ed.", F. Lifshitz (Ed.), Marcel Dekker, Inc. New York, 1990, 259-291.
3. de Peretti, E. and Forest, MG., "Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: evidence for testicular production", *J. Clin. Endocrinol. Metab.*, 1978, 47:572-577.
4. Lashansky, G., et. al., "Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation", *J. Clin. Endocrinol. Metab.*, 1991, 73:674-686.
5. Zumoff, B., et. al., "Sex differences in the twenty-four-hour mean plasma concentrations of dehydroisoandrosterone (DHA) and dehydroisoandrosterone sulfate (DHAS) and the DHA to DHAS ratio in normal adults", *J. Clin. Endocrinol. Metab.*, 1980, 51:330-333.
6. Pang S., "Late-onset adrenal steroid 3 beta-hydroxysteroid dehydrogenase deficiency. I. A cause of hirsutism in pubertal and postpubertal women", *J. Clin. Endocrinol. Metab.*, 1985, 60:428-39.

ASSAY PRINCIPLE

The DetectX[®] Dehydroepiandrosterone sulfate Immunoassay kit uses a specifically generated antibody to measure Dehydroepiandrosterone sulfate (DHEA-S) in serum, plasma, urine, and saliva samples, and in fecal extracts. The kit will also quantitatively measure DHEA-S present in tissue culture media samples. Please read the complete kit insert before performing this assay. A DHEA-S standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture sheep antibodies. A DHEA-S-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to DHEA-S to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound DHEA-S-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the Dehydroepiandrosterone sulfate in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
17-Hydroxyprogesterone EIA Kits	K053-H1/H5
Allopregnanolone EIA & CLIA Kits	K044-H1/H5, K044-C1/C5
Corticosterone EIA & CLIA Kits	K014-H1/H5, K014-C1/C5
Cortisol EIA & CLIA Kits	K003-H1/H5, H1W/H5W
Cortisone EIA & CLIA Kits	K017-H1/H5, K017-C1/C5
Estradiol Non-Invasive & Serum EIA Kits	K030-H1/H5, KB30-H1/H5
Estrone EIA Kits	K031-H1/H5
Progesterone EIA Kits	K025-H1/H5
PGFM (13,14,Dihydro-15-keto-PGF_α) EIA Kits	K022-H1/H5
Testosterone EIA Kits	K032-H1/H5



SUPPLIED COMPONENTS

Coated Clear 96 Well Plate

Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.
Kit K054-H1 **or** -H5 1 **or** 5 Each Catalog Number X061-1EA

Dehydroepiandrosterone sulfate (DHEA-S) Standard

Dehydroepiandrosterone sulfate at 1,200 ng/mL in a special stabilizing solution.
Kit K054-H1 **or** -H5 70 μ L **or** 350 μ L Catalog Number C201-70UL **or** -350UL

DetectX[®] Dehydroepiandrosterone sulfate (DHEA-S) Antibody

A sheep polyclonal antibody specific for dehydroepiandrosterone sulfate
Kit K054-H1 **or** -H5 3 mL **or** 13 mL Catalog Number C199-3ML **or** -13ML

DetectX[®] Dehydroepiandrosterone sulfate (DHEA-S) Conjugate

A dehydroepiandrosterone sulfate-peroxidase conjugate in a special stabilizing solution.
Kit K054-H1 **or** -H5 3 mL **or** 13 mL Catalog Number C200-3ML **or** -13ML

Assay Buffer Concentrate

A 5X concentrate that must be diluted with deionized or distilled water.
Kit K054-H1 **or** -H5 28 mL **or** 55 mL Catalog Number X065-28ML **or** -55ML

Wash Buffer Concentrate

A 20X concentrate that must be diluted with deionized or distilled water.
Kit K054-H1 **or** -H5 30 mL **or** 125 mL Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K054-H1 **or** -H5 11 mL **or** 55 mL Catalog Number X019-11ML **or** -55ML

Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**
Kit K054-H1 **or** -H5 5 mL **or** 25 mL Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K054-H1 **or** -H5 1 **or** 5 Each Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Ethanol or methanol for extraction of dried fecal samples.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate must be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers' Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.



SAMPLE TYPES

This assay has been validated for serum, plasma, saliva, urine, dried fecal extracts and for culture media samples. Samples containing visible particulate should be centrifuged prior to using. Dehydroepiandrosterone sulfate can be assayed in solid sample types by using one of the extraction protocols available on our website at: www.arborassays.com/resources/#protocols.

DHEA-S is identical across all species and we expect this kit to measure DHEA-S from all sources. The end user should evaluate recoveries of DHEA-S in other sample matrices being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

The minimum dilution for human serum and plasma samples is 1:2, but due to the high sample concentration most samples will have to be diluted at least 1:100 with diluted Assay Buffer. For measurement of DHEA-S in non-human samples it is recommended that the end user carry out a preliminary dilution series to determine the correct dilution for their samples.

Urine Samples

Urine samples must be diluted at least 1:2 with diluted Assay Buffer, but due to the high sample concentration most samples will have to be diluted at least 1:100 with diluted Assay Buffer. For measurement of DHEA-S in non-human samples it is recommended that the end user carry out a preliminary dilution series to determine the correct dilution for their samples.

For comparison to creatinine as a urine volume marker please see our NIST-calibrated 2 and 10 plate Urinary Creatinine Detection kits, K002-H1 and K002-H5.

Saliva Samples

Saliva samples must be diluted at least 1:2 with diluted Assay Buffer. A saliva collection and clarification protocol is available on our website at: www.arborassays.com/resources/#protocols.

Dried Fecal Samples

We have a detailed Extraction Protocol available on our website at: www.arborassays.com/resources/#protocols. The ethanol concentration in the final Assay Buffer dilution added to the well must be < 5%.

Culture Media

For measuring DHEA-S in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

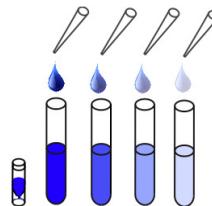
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Standard Preparation

Label test tubes #1 through #5. Pipet 380 μL of Assay Buffer into tube #1 and 160 μL into tubes #2 to #5. **The Dehydroepiandrosterone sulfate (DHEA-S) stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 20 μL of the DHEA-S stock solution to tube #1 and vortex completely. Take 40 μL of the DHEA-S solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #5. The concentration of DHEA-S in tubes 1 through 5 will be 60,000, 12,000, 2,400, 480 and 96 pg/mL .



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5
Assay Buffer (μL)	380	160	160	160	160
Addition	Stock	Std 1	Std 2	Std 3	Std 4
Vol of Addition (μL)	20	40	40	40	40
Final Conc (pg/mL)	60,000	12,000	2,400	480	96



ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine DHEA-S concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4°C.
2. Pipet 50 µL of samples or standards into wells in the plate.
3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer into the maximum binding (BO or Zero standard) wells.
5. Add 25 µL of the DetectX® Dehydroepiandrosterone sulfate Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Dehydroepiandrosterone sulfate Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 20% lower.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate Dehydroepiandrosterone sulfate concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.



Or use the online tool from MyAssays to calculate the data:

[www.myassays.com/arbor-assays-dehydroepiandrosterone-sulfate-\(dhea-s\)-eia-kit.assay](http://www.myassays.com/arbor-assays-dehydroepiandrosterone-sulfate-(dhea-s)-eia-kit.assay)



TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Dehydroepiandrosterone sulfate (DHEA-S) Conc. (pg/mL)
NSB	0.065	0	-	-
Standard 1	0.141	0.076	10.9	60,000
Standard 2	0.266	0.201	28.7	12,000
Standard 3	0.463	0.398	56.9	2,400
Standard 4	0.641	0.576	82.3	480
Standard 5	0.725	0.660	94.3	96
B0	0.765	0.700	100	0
Sample 1	0.308	0.243	34.7	8,208
Sample 2	0.413	0.348	49.6	3,580

Always run your own standard curve for calculation of results. Do not use this data.

Conversion Factor: 100 pg/mL of Dehydroepiandrosterone sulfate is equivalent to 256.1 pM.



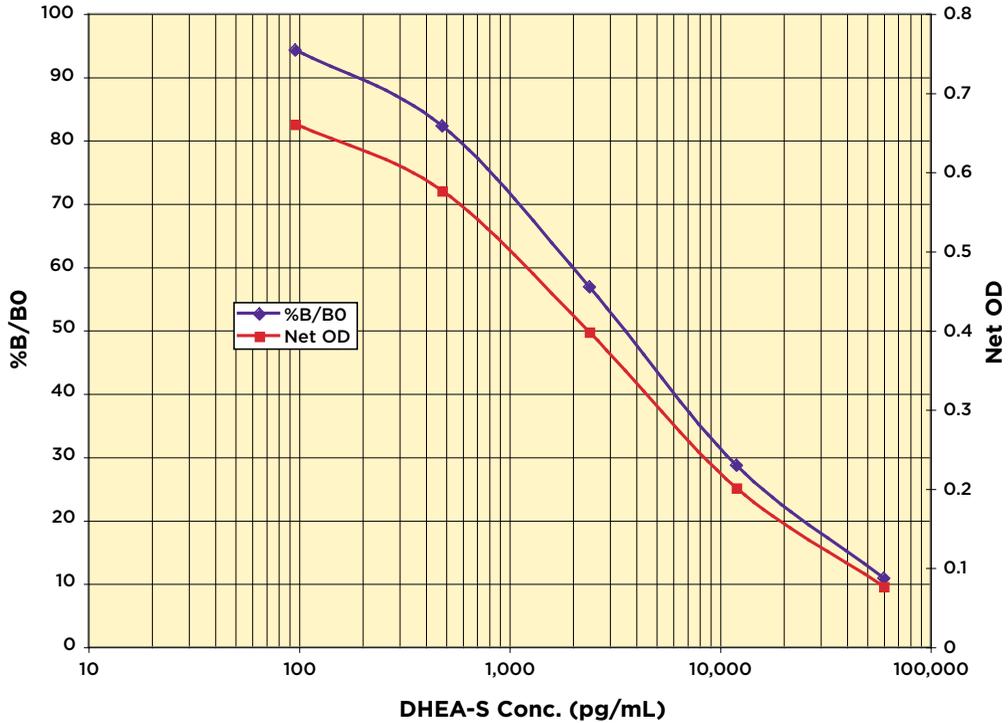
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EXPECT ASSAY ARTISTRY

Typical Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for eighteen wells run for each of the BO and standard #5. The detection limit was determined at two (2) standard deviations from the BO along the standard curve.

Sensitivity was determined as 90.9 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human serum sample.

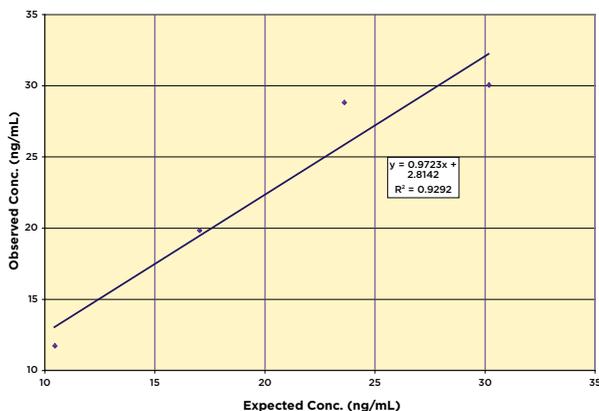
Limit of Detection was determined as 75.6 pg/mL.

Linearity

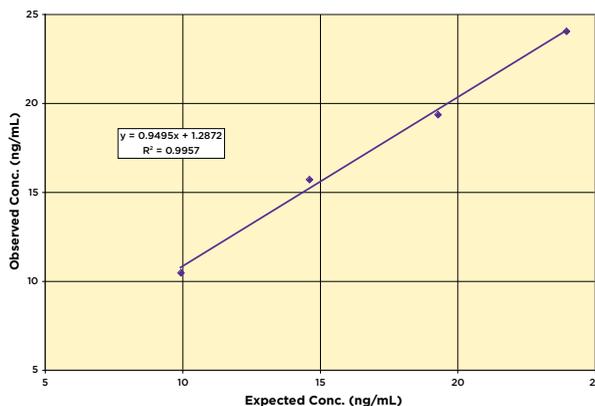
Linearity was determined using human serum and urine samples, by taking samples with a high known DHEA-S concentration and a lower DHEA-S concentration and mixing them in the ratios given below. The measured DHEA-S concentrations were compared to the expected values based on the ratios used.

High Sample	Low sample	Expected Conc. (ng/mL)		Observed Conc. (ng/mL)		% Recovery	
		Serum	Urine	Serum	Urine	Serum	Urine
80%	20%	30.20	23.96	30.02	24.04	99.4	100.3
60%	40%	23.62	19.29	28.80	19.34	121.9	100.3
40%	60%	17.05	14.62	19.82	15.70	116.2	107.4
20%	80%	10.47	9.95	11.70	10.46	111.8	105.2
Mean Recovery						112.3%	103.3%

Serum Linearity



Urine Linearity



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EXPECT ASSAY ARTISTRY

Intra Assay Precision

Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated DHEA-S concentrations were:

Sample	DHEA-S Conc. (pg/mL)	%CV
1	8,148	4.6
2	3,692	6.1
3	920.9	10.3

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in eighteen assays run over multiple days by four operators. The mean and precision of the calculated DHEA-S concentrations were:

Sample	DHEA-S Conc. (pg/mL)	%CV
1	8,567	5.2
2	3,794	8.4
3	932.2	11.5

SAMPLE VALUES

Nineteen serum samples were tested in the assay and adjusted neat concentrations of DHEA-S ranged from 438.5 to 5,879 ng/mL with a mean of 3,177 ng/mL.

Seven plasma samples were tested in the assay and adjusted neat concentrations of DHEA-S ranged from 299.9 to 5,847 ng/mL with a mean of 2,105 ng/mL.

Nine urine samples were tested in the assay and adjusted neat concentrations of DHEA-S ranged from 371.2 to 2,555 ng/mL with a mean of 942.4 ng/mL.

Five saliva samples were tested in the assay and adjusted neat concentrations of DHEA-S ranged from 0.170 to 8.24 ng/mL with a mean of 3.93 ng/mL.

Three fecal extracts were tested in the assay and adjusted neat concentrations of DHEA-S ranged from 5.40 to 429 pg/mg with a mean of 278 pg/mg.

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)	Steroid	Cross Reactivity (%)
DHEA-S	100	Progesterone	0.2
DHEA	162.0	Estrone	0.1
Epiandrosterone	44.5	17OH-Progesterone	0.1
Androsterone	28.4	17OH-Pregnenolone	< 0.1
Androstenedione	15.2	Aldosterone	< 0.1
DHT	0.5	Corticosterone	< 0.1
Adrenosterone	0.4	Cholesterol	< 0.1
Testosterone	0.4	Estradiol	< 0.1
Desoxycorticosterone	0.2	Pregnenolone	< 0.1

In serum the relative level of DHEA are typically between 1 and 0.1% of the DHEA-S concentration. The cross reactivity to DHEA with the assay will contribute to an increase in measured DHEA-S concentrations of less than 2%.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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