

FOR PROFESSIONAL USE ONLY

# Dried Blood Spot (DBS) Prolactin ELISA

**RUO**

## AL-1016

### INTENDED USE

The Prolactin (DBS) Enzyme-Linked Immunosorbent Assay (ELISA) Kit provides materials for the quantitative measurement of prolactin in human dried blood spot. This assay is intended for *Research Use only*.

### SUMMARY AND EXPLANATION

Prolactin is a 198-amino acid, 23 kDa polypeptide hormone secreted in significant amounts by the anterior pituitary gland. The prolactin molecule has extensive sequence homology with growth hormone and placental lactogen<sup>1</sup>. Recent studies have revealed molecular heterogeneity for prolactin in both pituitary extracts and blood<sup>2,3</sup>. Prolactin secretion is regulated by complex mechanisms involving neurotransmitters and endocrine hormones. Many of the regulatory pathways involve hypothalamic secretion of dopamine, which inhibits prolactin secretion<sup>4</sup>. Prolactin, in synergy with estrogen, plays an important physiologic role in the initiation and maintenance of mammary gland growth and lactation in humans<sup>1,4</sup>. In addition, prolactin may have effects on cell growth in other tissues and on immune function. Particularly when present in high concentrations, prolactin may have inhibitory effects on gonadal function. Prolactin is present in several body fluids, including plasma, amniotic fluid, milk, mucosal secretions, and cerebrospinal fluid. Relative elevations in plasma prolactin concentrations occur during ovulation, pregnancy, nursing and stress<sup>1,2,4</sup>. Abnormal elevations in plasma prolactin levels, or hyperprolactinemia, can occur as a result of pituitary adenomas, and may also be seen in other anatomic and traumatic abnormalities involving the pituitary gland (e.g. tumors, surgery, trauma), as a consequence of certain pharmacologic agents, and primary hypothyroidism. Low prolactin levels, or hypoprolactinemia, is observed in cases of hypopituitarism<sup>5,6</sup>.

### PRINCIPLE OF THE TEST

The DBS Prolactin ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and extracted dried blood spot samples are added to Prolactin antibody coated microtiter wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated Prolactin antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The biotin-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of Prolactin in the samples and calibrators.

### MATERIALS SUPPLIED

#### CAL-199A Prolactin Calibrator A

One vial, 2 mL, labeled Prolactin Cal. A, containing 0 ng/mL Prolactin in protein based buffer and Pro-Clean 400. Store unopened at 2-8°C until the expiration date.

**Standardization Note:** The Prolactin calibrators are traceable to the World Health Organization International preparation NIBSC code 83/573 version 5.0.

#### CAL-199B - CAL-199E Prolactin Calibrators B – E (Lyophilized)

Four vials, labeled B-E, containing concentrations of approximately 2.2 – 366.0 ng/mL Prolactin in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-E with **1 mL deionized water**. Solubilize, mix well, and use after reconstitution. Aliquot and freeze in plastic vials immediately for multiple use and discard after the run. Avoid repeated freeze thaws.

#### CTR-199-I & CTR-199-II Prolactin Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high Prolactin concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with **1 mL deionized water**. Solubilize, mix well, and use after reconstitution. Aliquot and freeze immediately in plastic vials for multiple use and discard after run. Avoid repeated freeze thaws.

#### PLT-199 Prolactin Antibody Coated Microtitration strips

One strip-holder, containing 12 strips and 96 microtitration wells with anti-Prolactin antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

#### ASB-199 Prolactin Assay Buffer

One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

#### BCR-1016 Prolactin Biotin Conjugate Ready-To-Use (RTU)

One bottle, 12 mL containing biotinylated anti-Prolactin antibody in a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

#### SAR-1016 Prolactin Streptavidin-Enzyme Conjugate-Ready-To-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store at 2-8°C until expiration date.

#### EXB-129 Extraction Buffer/Sample Diluent

One bottle, 45 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

*Note: Additional bottles of EXB-129, Extraction Buffer/Sample Diluent can be ordered if higher dilution is required.*

**TMB-100 TMB Chromogen Solution**

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

**STP-100 Stopping Solution**

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2-30°C until expiration date.

**WSH-100 Wash Concentrate A**

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate absorbance reader capable of absorbance measurement at 450 nm, 405 nm, and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Repeater Pipette
8. Disposable 12 x 75 mm culture tubes
9. Tight fitting 12 x 75 mm tube racks
10. Ahlstrom 226 or Whatman 903 (Protein Saver Card)
11. DBS 5/16" (7.9 mm) round puncher. For manual punching Punchline catalog number 53700 from McGill Incorporated can be used.

**WARNINGS AND PRECAUTIONS**

*For Research Use Only. Not for use in diagnostic procedures.*

The following precautions should be observed:

- a) Follow good laboratory practice.
- b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
- c) Handle and dispose of all reagents and material in compliance with applicable regulations

**WARNING: Potential Biohazardous Material**

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5<sup>th</sup> Edition, 2007.<sup>7</sup>

**WARNING: Potential Chemical Hazard**

Some reagents in this kit contain Pro-Clean 400 and Sodium azide<sup>8</sup> as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

**SAMPLE COLLECTION AND PREPARATION**

**Dried Blood Spot** is the recommended sample type.

1. Use with capillary blood samples collected and dried on filter paper (Ahlstrom 226 or Whatman 903 Protein Saver Card) according to the standard procedures established for blood collection on filter paper.
2. Wipe away the first blood drop and apply surface of the first filter paper circle to the next large drop of blood, allowing the blood to fill

and completely saturate the circle. Note: **Alternatively, the filter paper can be spotted by adding 60 µL of whole blood from K2- EDTA tubes.**

3. Never use the front as well as back of the paper to fill the circle.
4. Fill at least two circles and if possible, all circles with blood.
5. After collection, dry the blood impregnated filter papers for **2-4 hours** in a horizontal position at room temperature.
6. The dried filter paper blood spots should be stored in a low permeability re-sealable pouch at 2-8°C with a desiccant for up to 1 week or frozen at -20°C or lower for up to 3 months. *Note: The use of desiccant and vacuum packing to protect from moisture is highly recommended.*

**PROCEDURAL NOTES**

1. A thorough understanding of this package insert is necessary for successful use of the DBS Prolactin ELISA assay. It is the user's responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature ( $23 \pm 2^\circ\text{C}$ ) before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents or contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, Sodium Azide, Hypochlorous acid, and aromatic Chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

**PREPARATION OF REAGENTS**

1. **Prolactin Calibrators B-E and Prolactin Controls I & II:** Tap and reconstitute Prolactin Calibrators B-E and Prolactin Controls I & II each with **1 mL** deionized water and solubilize for 15 minutes, vortex and use. **Note:** Dilute reconstituted calibrator B with a factor of 1:3. In the case of duplicates use the following: In a separate vial add 150 µL of Reconstituted Calibrator B to 300 µL Calibrator A (0 ng/mL). Vortex and add in calibration curve.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature ( $23 \pm 2^\circ\text{C}$ ) when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

**DBS Extraction Procedure**

**Extraction of Prolactin from dried blood spots should be performed on the same day prior to testing.**

NOTE: All blood spots should be inspected for quality.

- Do not use spots if the circle is not filled and impregnated with blood.
- Do not use irregular shaped spots, spots that are not impregnated throughout, or spots with multiple spotting.

- Do not use spots that have not been properly dried.

- Label two 12 X 75 culture tubes for each unknown dried blood sample.
- Punch out **two filter paper discs (7.9 mm)**, impregnated with the unknown dried blood specimen, onto a clean surface and transfer the discs using clean tweezers into the corresponding tube.
- Alternatively, punch out the paper disc directly into the culture tube using the commercially available automated punchers.
- Add **450 µL of the Extraction Buffer (EXT-129)** to each tube, vortex well.
- Place the tubes in a tight-fitting tube rack and incubate the tubes, shaking at a slow speed (500 - 600 rpm) at room temperature for 60 minutes.
- Transfer the liquid from one tube into the corresponding second labeled tube. Leave the blood spot in the initial tube.
- The blood extract is now ready for analysis.
- The extracted sample (without the extracted blood spot) is stable for up to 7 days at -20°C.
- Use the **calibrator assignment for two spots** as mentioned in the calibration card for plotting the calibration curve.

### ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

- Reconstitute Prolactin Calibrators B-E and Prolactin Controls I & II each with **1 mL** deionized water as per the "Preparation of Reagents" section of this package insert. Solubilize for 10 minutes. Mix well by gentle vortex.
- Label the microtitration strips to be used.
- Pipette **20 µL** of the **Calibrators and Controls** (Including B/3 Refer step 1 in Preparation of Reagents) to the appropriate wells and add **100 µL of the Prolactin Assay Buffer** to calibrators and controls wells using a repeater pipette.
- Pipette **120 µL of the Extracted DBS samples** (see DBS extraction procedure) to the appropriate wells. **Note: Do not add Prolactin Assay Buffer to the extracted sample wells.**
- Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
- Aspirate and wash each strip **5 times (350 µL/per well)** with Wash Solution using an automatic microplate washer.
- Add **100 µL of the Prolactin Biotin Conjugate Ready-To-Use (RTU)** to each well using a repeater pipette.
- Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
- Aspirate and wash each strip **5 times (350 µL/per well)** with **Wash Solution** using an automatic microplate washer.
- Add **100 µL of the Prolactin Streptavidin-Enzyme Conjugate-RTU** to each well using a repeater pipette.
- Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **15 minutes** at room temperature (23 ± 2°C).
- Aspirate and wash each strip **5 times (350 µL/per well)** with the **Wash Solution** using an automatic microplate washer.
- Add **100 µL of the TMB chromogen** solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
- Incubate the wells, shaking at **600-800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).  
NOTE: Visually monitor the color development to optimize the incubation time.
- Add **100 µL of the Stopping Solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.

**NOTE:** Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to

dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.

### RESULTS

**NOTE:** The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log Prolactin concentration on X-axis** using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.

- Calculate the mean optical density (OD) for each calibrator, control, or Unknown.
- Optimum results can be obtained at incubation temperature of (23 ± 2°C).
- Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Prolactin concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
- Determine the Prolactin concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Prolactin concentrations.
- Any sample reading higher than the highest Calibrator should be appropriately further diluted with the 0 ng/mL (Cal. A) and re-assayed.
- Any sample reading lower than the analytical sensitivity should be reported as such.

### LIMITATIONS

The reagents supplied in this kit are optimized to measure Prolactin levels in human DBS. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.<sup>9</sup>

The DBS Prolactin ELISA results should be interpreted with respect to the total clinical presentation of the patient, including symptoms, clinical history, data from additional tests, and other appropriate patient examination information.

### QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Prolactin ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Prolactin controls are printed on the **Calibration Card**.
- A full calibration curve, low, and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

### REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean Absorbance (OD)	Conc. (ng/mL)
A1, A2	Calibrators A	0.010 (Blank)	0
B1, B2	B/3	0.048	2.2
C1, C2	B	0.15	6.6
D1, D2	C	0.53	22.8
E1, E2	D	1.7	84
F1, F2	E	3.8	366

**CAUTION:** The above data must not be employed in lieu of data obtained by the user in the laboratory.

**ANALYTICAL CHARACTERISTICS**

All performance characteristics were performed with serum except for Method Comparison.

**Limit of Blank (LOB):**

The Limit of Blank was 0.132 ng/mL, calculated from a minimum of n=18 measurements of calibrator A.

**Limit of Detection (LoD):**

The lowest amount of Prolactin in a sample that can be detected with a 95% probability (n=24) is 0.4 ng/mL. The value was determined by processing six samples in the range of 1.5 to 5.0 ng/mL following CLSI EP17 guidelines. Eight assay runs were performed over four days with samples run in triplicates per run.

**Imprecision:**

Reproducibility of the Prolactin ELISA assay was determined in a study using three samples. The study included a total of 6 assays, 3 replicates of each per assay (n=18). Representative data were calculated and are presented in the following table.

Sample	Mean conc. (ng/mL)	Within run		Total	
		SD	%CV	SD	%CV
Pool-1	7.131	0.198	2.78%	0.240	3.37%
Pool-2	30.206	0.645	2.13%	0.906	3.00%
Pool-3	103.551	3.582	3.46%	7.800	7.53%

**Linearity:**

Multiple dilutions of the three serum samples containing various Prolactin levels were diluted with Calibrator A. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
Sample 1	Neat Value	64.299		
	2	32.150	35.255	
	4	16.075	17.431	111%
	8	8.037	9.096	
	16	4.019	4.506	
Sample 2	Neat Value	58.410		
	2	29.205	30.598	111%
	4	14.603	16.361	
	8	7.301	8.459	
Sample 3	Neat Value	31.911		
	2	15.956	17.023	111%
	4	7.978	9.155	

**Recovery:**

Known amounts of Prolactin were added to five serum samples containing different levels of endogenous Prolactin. The concentration of Prolactin was determined before and after the addition of exogenous Prolactin and the percent recovery was calculated.

Sample	Endogenous Conc. (ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
S1	4.692	16.657	16.716	100%
		28.623	27.093	95%
		40.588	37.617	93%
S2	3.166	15.208	16.589	109%
		27.249	26.121	96%
		39.291	39.301	100%
S3	9.288	21.024	20.333	97%
		32.759	30.274	92%
		44.495	39.754	89%
S4	53.112	62.656	59.917	96%
		72.201	68.460	95%
		81.745	78.007	95%
S5	13.176	24.717	23.452	95%
		36.258	33.987	94%
		47.800	38.904	81%

**Analytical Specificity:****Cross-Reactivity**

FSH, LH, hCG, and TSH when tested at twice the physiological dose were below the detection limit of the assay. Monoclonal antibody pair used in the assay detects human, bovine and monkey Prolactin.

**Hook Effect:**

There is no high-dose hook effect at Prolactin concentrations up to 3000 ng/mL.

**Expected Value:**

The expected Prolactin concentration ranges (95% CI) for Prolactin in males and females were calculated and listed in the table below.

Population	Age Range (Yrs.)	No. of Specimens	Median Prolactin (ng/mL)	95% CI Range Prolactin (ng/mL)
Male	18-89	50	20.8	4.9-58.2
Female	15-91	54	25.6	6.3-236.9

The expected Prolactin concentration ranges (95% CI) for Prolactin in pregnant females in first and second trimesters were calculated and listed in the table below.

Population	Age Range (Yrs.)	Gest. Age (wks., days)	No. of Specimens	Median Prolactin Conc. (ng/mL)	95% CI Range Prolactin (ng/mL)
1st Trimester	17-40	11,5 - 14,1	19	52.9	31.9-256.1
2nd Trimester	19-42	15,2 - 20,1	20	105.8	37.1-237.6

**Interference:**

When potential interferents (Hemoglobin, biotin, bilirubin, and intralipids) were added at least two times their physiological concentration to control sample, Prolactin concentration were within  $\pm 10\%$  of the control as represented in the following table. This study was based on NCCLS EP-7 to serum matrix added.

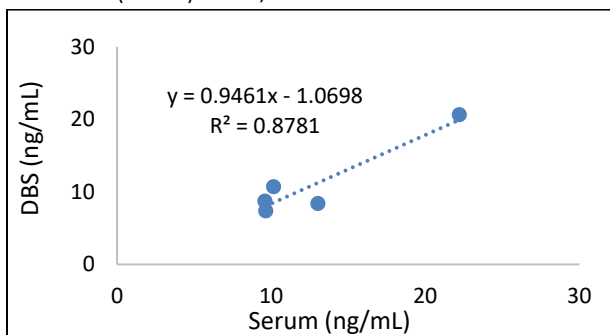
Interferents	Interferent Dose	Analyte Conc. (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1 mg/mL	12.24	11.86	-3.1
	0.5 mg/mL	13.03	12.78	-1.9
	0.1 mg/mL	12.40	12.40	0.0
Hemoglobin	1 mg/mL	15.63	15.32	-2.0
	0.5 mg/mL	16.59	16.65	0.4
	0.1 mg/mL	17.54	16.81	-4.1
Biotin	1200 ng/mL	12.97	12.53	-3.4
	600 ng/mL	12.85	12.95	0.8
	200 ng/mL	12.57	12.91	2.7
Biotin	1200 ng/mL	15.78	15.74	-0.3
	600 ng/mL	16.41	16.93	3.1
	200 ng/mL	17.63	18.21	3.3
Intralipids	20 mg/mL	18.11	18.52	2.3
	10 mg/mL	18.54	18.52	-0.1
	5 mg/mL	18.72	18.67	-0.2
Intralipids	20 mg/mL	21.59	22.53	4.3
	10 mg/mL	24.12	24.80	2.8
	5 mg/mL	25.28	23.56	-6.8
Bilirubin	0.66 mg/mL	13.87	13.52	-2.6
	0.2 mg/mL	16.82	17.23	2.4
Bilirubin	0.66 mg/mL	15.31	15.56	1.7
	0.2 mg/mL	20.97	21.81	4.0

**Method Comparison:**

**Serum vs Dried Blood Spot:** Five matched serum samples were compared with dried blood spot in Prolactin ELISA Assay (AL-199).

Analysis of the results yielded the following regression:

$$\text{DBS} = 0.95 (\text{Serum}) - 1.07, \text{Correlation } r = 0.937$$



9. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

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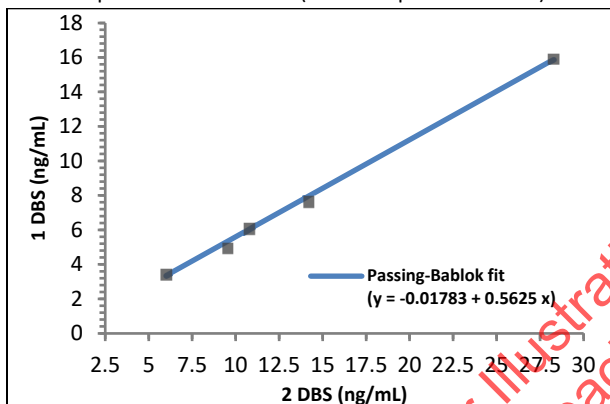
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**One versus Two Dried Blood Spots:** Extraction was performed using matched one versus two dried blood spots. Passing Bablok analysis of the result yielded the following regression:

$$\text{One DB Spot Extracted} = 0.56 (\text{Two DB Spots Extracted}) - 0.018$$

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