

Standardized and Efficient Automated Washing of LUNARIS™ BioChips on BioTek 405 Select Microplate Washer

A semi-automated workflow for processing of LUNARIS™ multiplex assays in life science research and routine lab procedures

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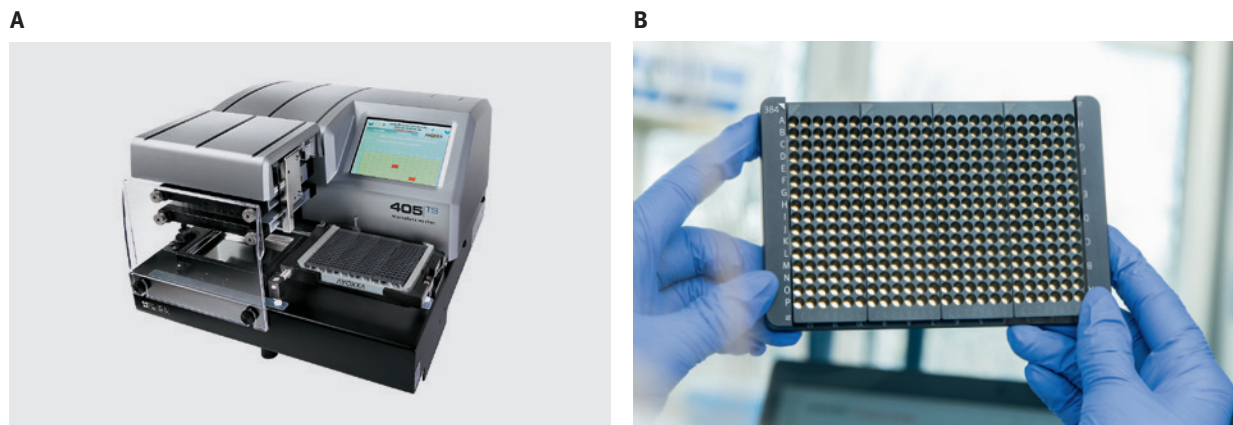


Figure 1. (A) BioTek 405 Select Microplate Washer, (B) LUNARIS™ BaseFrame equipped with 4 × 96-well BioChips.

Abstract

Efficient microplate washing is an essential step in antibody-based protein assays. The LUNARIS™ multiplex assays use highly standardized and effective plate tapping and aspiration protocols. Expanding the applications spectrum of this technology further, we present a washing protocol using state-of-the-art liquid handling technology that is easily implemented on commercial 384-well microplate washers like the BioTek 405 Select model. By reducing hands-on time and operator variability, this standardized semi-automated workflow ensures speed and results reproducibility in labs with highly routinized procedures. Furthermore, the unique combination of LUNARIS™ and liquid handling technologies brings added efficiency into life science labs processing large numbers of samples without compromising small sample volume or assay sensitivity and robustness.

Sensitivity:

- > LoD in single-digit pg/mL range with 3–4 log scale quantifiable range

Robustness:

- > Uniformity of signal over entire 384-well plate <14% CV

Performance:

- > Within-Run Precision <10% CV and Within-Run Accuracy in 70–130% range
- > Between-Run Precision <20% CV and Between-Run Accuracy in 70–130% range

Introduction

LUNARIS™, AYOXXA's proprietary beads-on-a-chip multiplex assay platform enables basic to clinical research, across drug development to clinical application¹. The platform allows simultaneous quantification of up to 12 biomarkers in a format compatible with 96- or 384-well ANSI-SBS* microplates. This plate-based format combined with the modular design of LUNARIS™ BioChips supports testing on 384-well microtiter plates allowing protein analysis of up to 160 samples in duplicates, considering the analysis of standards, blanks and controls.

The BioTek 405 Select Microplate Washer includes various features suited to a range of assay applications, from basic ELISA to sensitive cell and bead washing. Its Dual-Action™ manifold allows independent control of aspiration and dispense tubes, while an available built-in ultrasonic cleaner reduces assay failure, system downtime and maintenance efforts. Automatic buffer switching for up to four wash buffers is optional, and the washer can be controlled using the built-in keypad or via the Liquid Handling Control™ software on a PC.

The standard LUNARIS™ immunoassay protocol includes manual washing using a multichannel pipet for convenience, delivering sensitivities in single-digit pg/mL concentrations with a quantifiable range of 3–4 log scales, a coefficient of variation (CV ≤20%) and recovery rates between 70–130%. However, to meet the requirements of today's busy life science research labs, a semi-automated washing procedure is most valuable to keep up with the workload without compromising assay robustness.

Accuracy and precision in microplate washing is essential for assay reproducibility and robustness. Here we describe an adaptation of the LUNARIS™ protocol that employs the BioTek 405 Select Microplate Washer (BioTek Instruments, USA) to automate the washing steps and enable high-throughput analysis.



Figure 2. Schematic of the LUNARIS™ multiplex procedure

(A) The LUNARIS™ assay follows the classical sandwich ELISA principle. Samples are added to each well of the BioChip, and targeted analytes are captured by the antibody-coated beads during incubation. The wells are washed, and the captured analytes are labeled with biotinylated detection antibodies and streptavidin-phycoerythrin (SA-PE). The plates are air-dried and subsequently imaged with the LUNARIS™ Reader (B) to quantify the fluorescence signal on the beads. (C) The LUNARIS™ Analysis Suite Software provides a tailored report with detailed information on the concentration of target proteins in each sample.

* ANSI-SBS: Microtiterplate footprint dimensions recommended by the Society of Biomolecular Screening www.slas.org

Material and Methods

To evaluate and optimize the washing protocol, the LUNARIS™ Human 11-plex Cytokine Kit was used (Cat. No. LHCY-20110F). Four LUNARIS™ BioChips were assembled to fill up the LUNARIS™ BaseFrame. The calibration curves were generated according to the kit handbook. Briefly, seven standards were prepared by serial dilution (1:4) of the Human Cytokine Standard using Assay Diluent 1. The standard contained the following analytes: IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF- α , and GM-CSF. Three known concentrations (low, medium and high) within the quantifiable range of the cytokine standard curves were spiked into human serum samples (Seralab, UK) and used as controls.

The LUNARIS™ BioChips were first prewashed, and then 5 μ L each of standard, blank and spiked control was loaded into the appropriate wells (Figure 3A). After an incubation of 3 hours at room temperature (RT), the plate was washed using the BioTek 405 Select Microplate Washer, as described in the washing instructions (Figure 3B). After washing, 10 μ L of detection antibody reagent was added and allowed to incubate for 60 minutes at RT. The plate was washed again, and then 10 μ L of SA-PE reagent was added. After a 30-minutes incubation, the final plate wash was performed. The plate was air-dried and imaged using the LUNARIS™ Reader 384 (Cat. No. LRS-202). The quantification of the readout was performed using the LUNARIS™ Analysis Suite (LAS) software (Cat. No. LAS-001).

To assess the reproducibility of the wash protocol, the precision and accuracy among the four BioChips constituting the 384-well plate were evaluated. The experiment used a single reagent lot^{1,2}. One BioChip was designated as one run, and Within- and Between-BioChip Precision and Accuracy were defined as Within- and Between-Run (WRP, BRP, WRA and BRA, see »Definitions« on page 6). The WRA and WRP were determined by measuring the controls, 1:2 diluted in Assay Diluent 1. For each control (low, medium and high spike-in concentration), five independent preparations were analyzed on each BioChip, each preparation in triplicate. The BRP and BRA were determined by measuring the controls over the four individual LUNARIS™ BioChips.

Automated plate washing was performed using the BioTek 405 Select Microplate Washer with a 96-well Dual-Action™ manifold. The specific parameters of the procedure are listed in Figure 3B and have been optimized for washing efficiency and assay precision³.

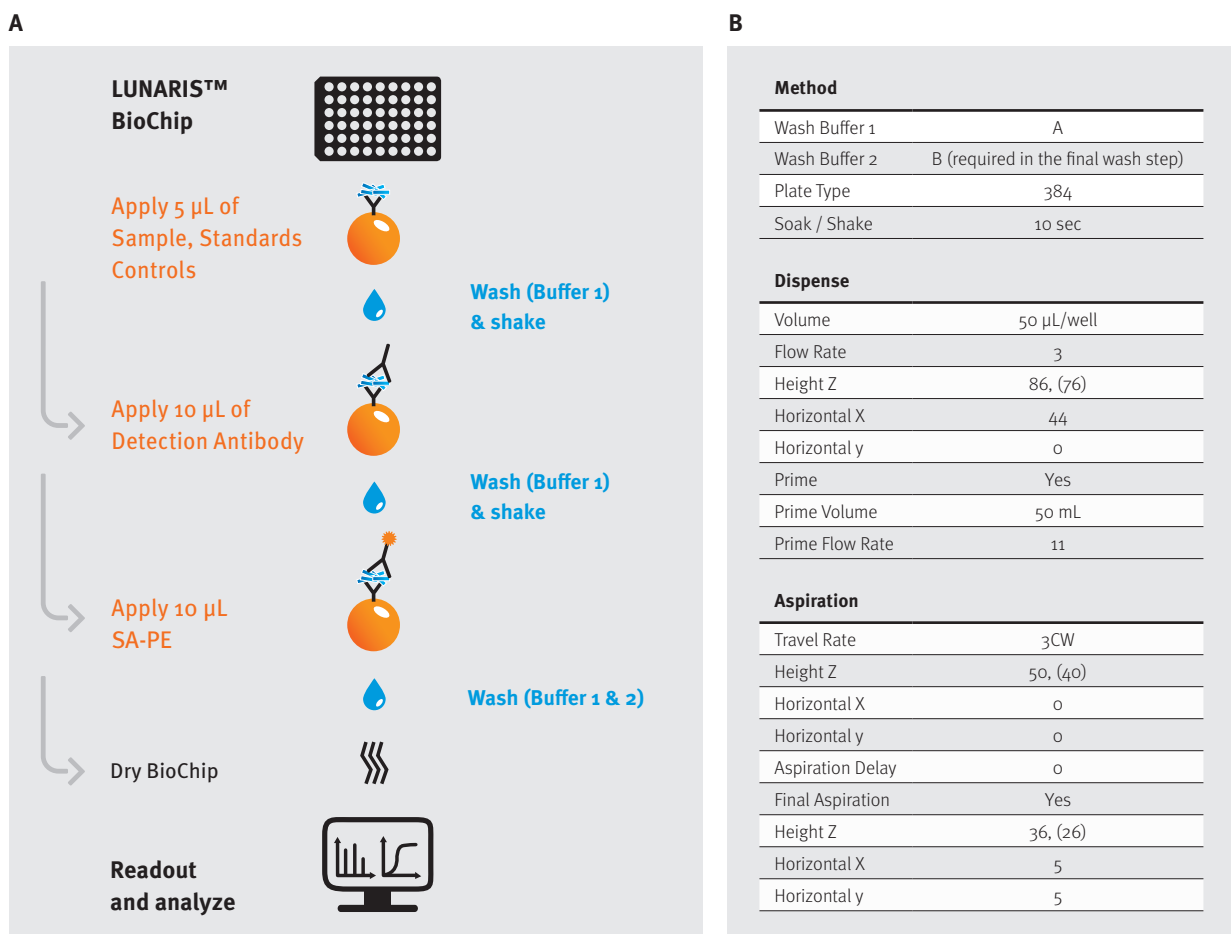


Figure 3. Washing procedure during LUNARIS™ assay workflow. (A) LUNARIS™ assay procedure and associated washing steps performed by BioTek 405 Select Microplate Washer. (B) The specific plate washing parameters for BioTek 405 Select TS & (Select) Microplate Washer models respectively.

Results and Discussion

The wash protocol using the BioTek Microplate Washer (Figure 3B) was optimized to ensure signal robustness and a desired well-to-well CV below 20%. The precision of the aspirate and dispense manifold was assessed on four 96-well BioChips forming a 384-well plate and by analyzing the measured signal using a constant standard concentration⁴. The homogeneous signal, measured across the four BioChips, is shown for the representative cytokine IL-10 (Figure 4A).

The inter-replicate CV of each cytokine was calculated per column, per row and across the entire plate (Figure 4B). For all cytokines analyzed, the observed mean inter-replicate CV was around 10% per column and per row. The inter-replicate CV across the plate ranged between 11–14%.

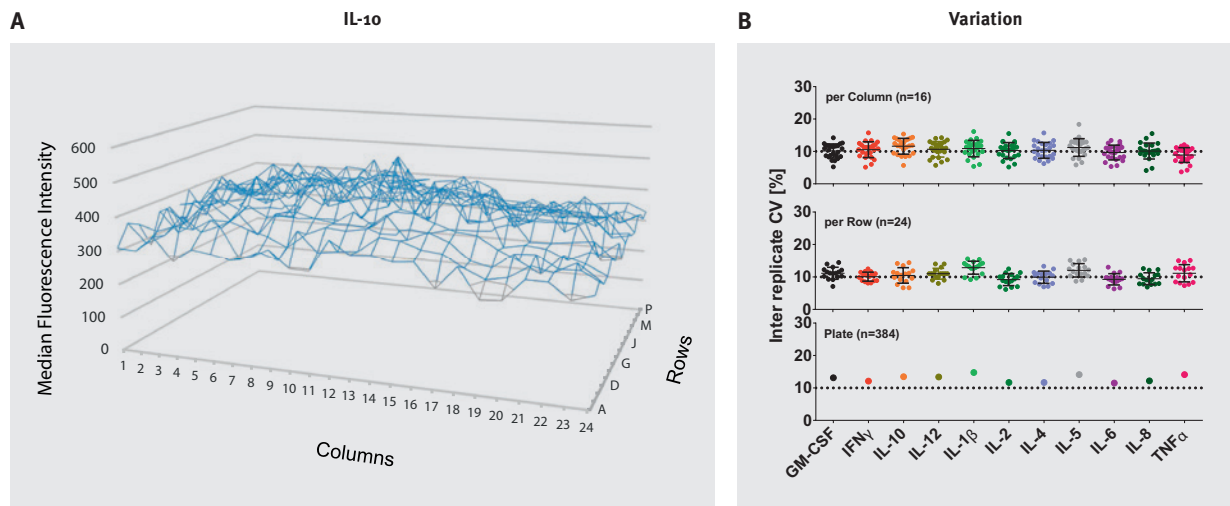


Figure 4. Variation on four 96-well BioChips (384-well plate) (A) 3-D surface plot of Median Fluorescence Intensity of representative IL-10, measured in each well (n=384 wells). (B) Inter-replicate CV of the signal measured for all cytokines on four 96-well BioChips, per column (24 columns, 16 wells each), per row (16 rows, 24 wells each) and over the entire plate (384 wells), represented in a scatter plot with mean percentage and standard deviation.

Once the automated wash protocol was established, the WRP, WRA, BRP and BRA were obtained by measuring three different concentrations of spiked controls on four 96-well LUNARIS™ Human 11-plex Cytokine Kit BioChips (see Materials and Methods). One BioChip was designated as one run. Therefore, the WRP, BRP, WRA and BRA were defined as Within- and Between-BioChip. For each BioChip, a single standard curve comprising all cytokines was generated to determine the sensitivities and analytical measurement ranges (Figure 5, Table 1).

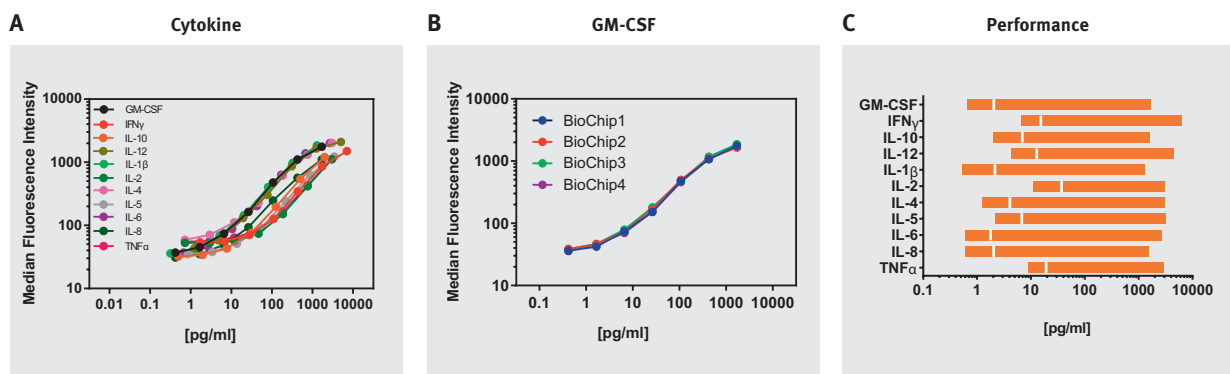


Figure 5. LUNARIS™ Human 11-plex Cytokine Kit Performance (A) Standard curves generated by diluting the Human Cytokine Standard 1:4 in Assay Diluent 1. (B) Comparison of GM-CSF standard curve from the four BioChips forming a 384-well plate. (C) Mean values of limit of detection (LoD left border), lower limit of quantification (LLOQ middle border) and upper limit of quantification (ULOQ right border) obtained from eight individual BioChips, constituting two 384-well plates.

Table 1. Sensitivity and quantifiable range of the automated washing procedure using the BioTek Microplate Washer and the LUNARIS™ Human Cytokine 11-plex Kit. Mean, minimum and maximum values of LoD, LLOQ, ULOQ and quantifiable range were calculated from analysis assays run on eight individual BioChips, constituting two 384-well plates, one plate per experimental day.

Analyte	LoD [pg/mL]			LLOQ [pg/mL]			ULOQ [pg/mL]			Log Quantifiable Range		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
GM-CSF	0.76	0.20	2.10	1.7	0.4	6.6	1700	1700	1700	3.2	3.2	3.2
IFN γ	6.58	2.40	25.80	12.0	6.8	27.3	6344	1750	7000	3.8	3.2	3.8
IL-10	2.01	0.50	4.70	5.4	0.5	7.8	1625	500	2000	3.2	2.7	3.3
IL-12	4.92	0.01	11.50	10.4	1.2	19.5	4531	1250	5000	3.6	3.1	3.7
IL-1 β	0.53	0.10	1.70	1.7	0.3	5.1	1300	1300	1300	3.1	3.1	3.1
IL-2	11.04	5.30	26.60	29.3	11.7	46.9	3000	3000	3000	3.5	3.5	3.5
IL-4	1.24	0.10	3.10	3.2	0.7	11.7	3000	3000	3000	3.5	3.5	3.5
IL-5	2.14	0.40	6.60	5.3	0.9	13.7	3172	875	3500	3.5	2.9	3.5
IL-6	0.61	0.02	1.60	1.4	0.7	2.6	2700	2700	2700	3.4	3.4	3.4
IL-8	0.61	0.10	2.10	1.7	0.4	6.6	1541	425	1700	3.2	2.6	3.2
TNF α	8.94	1.00	31.40	15.0	2.9	46.9	3000	3000	3000	3.5	3.5	3.5

The signals measured for the spiked controls were interpolated in the standard curves to determine WRP and BRP and WRA and BRA, as described above in Material and Methods. For all cytokines and concentrations analyzed, excellent WRP and BRP were observed on each and across the BioChips, with WRP mostly below 10% and BRP mostly below 15% (Figure 6 A–B)². WRA and BRA was consistently within the acceptance range of 70–130% over four BioChips (Figure 6 C–D).

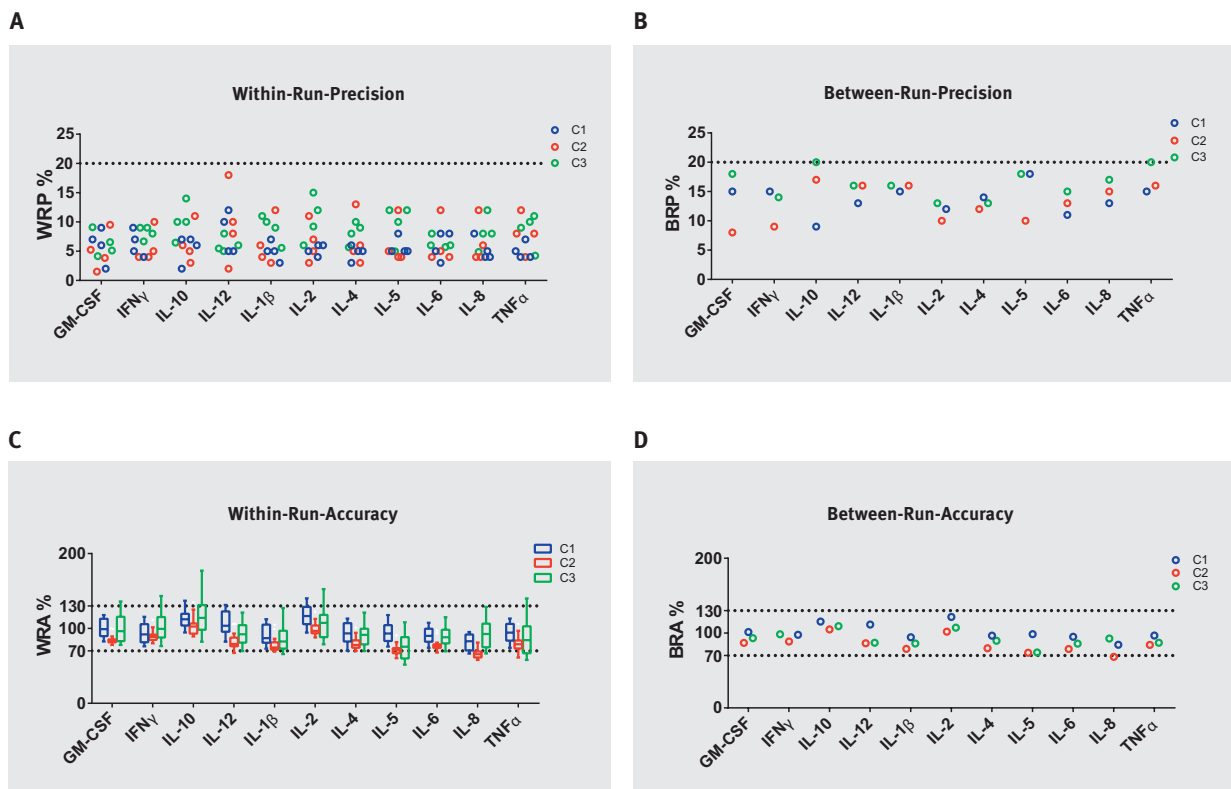


Figure 6. WRP, BRP, WRA and BRA of the automated washing procedure using the BioTek Microplate Washer and the LUNARIS™ Human 11-plex Cytokine Kit. Three known concentrations within the quantifiable range of the standard curves of all cytokines were spiked into human serum to produce the control samples (low, medium and high spike-in concentration, respectively C1, C2 and C3). (A) and (C) WRP and WRA were determined by measuring the controls from five independent preparations on each BioChip; each prepared control was analyzed in triplicate. (A) Scatter plot shows the WRP obtained per control sample, per BioChip, per analyte. (C) Box plot shows the WRA range measured, with median, min and max, per control, and per analyte. (B) and (D) BRP and BRA were obtained by measuring the controls over four BioChips and in triplicate.

Definitions

Assay precision	Percentage coefficient of variation (%CV) within (WRP) and between analytical runs (BRP). $\%CV = SD / \text{Mean} \times 100\%$, where SD is the standard deviation, and the mean corresponds to the back-calculated concentration of spiked control.
Assay accuracy	Percentage recovery to determine the closeness of measurements to the expected concentrations within (WRA) and between the runs (BRA). $\%Accuracy = \text{Measured concentration} / \text{Expected concentration} \times 100\%$.
LoD (Limit of Detection)	Lowest concentration of an analyte for which signals can be distinguished from the blanks ($2 \times$ standard deviation of the blank).
LLOQ (Lower limit of Quantification)	Lowest analyte concentrations detectable per analytical assay with the required precision of $\leq 20\%$ and accuracy of 70 to 130%.
ULOQ (Upper limit of Quantification)	Highest analyte concentrations detectable per analytical assay with the required precision of $\leq 20\%$ and accuracy of 70 to 130%.

This report summarizes the adaptation of the LUNARIS™ assay protocol using the BioTek 405 Select Microplate Washer. The procedure is robust and precise, allows biomarker detection in single-digit pg/mL range over 3-4 log scale quantifiable range. Thus the method is compatible with a semi-automated workflow needed for high-throughput applications.

For more information, please visit:

www.ayoxxa.com
www.biotek.com

References

1. Please find all recent scientific publications using AYOXXA's LUNARIS™ technology on our [website](#).
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LUNARIS™ Products are research use only, not for use in diagnostic procedures.

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