

Trouble Shooting Guide for the Argutus Medical EIAs

BIO60HEPA – Hepkit EIA
 BIO66NEPHA – Nephkit EIA
 BIO85 – Pi GST EIA
 BIO60HEPAS – High Sensitivity alpha GST EIA

BIO64RT – Rat Alpha GST EIA
 BIO76YB1 – Rat GST Yb1 EIA
 BIO89RPA1 – Rat RPA-1 EIA

Points to note before you start	<ul style="list-style-type: none"> ➤ Serum and Plasma Samples can be stored at -20°C for long term storage. Diluted samples should not be stored. Urine samples must be stored as per specific Instructions for use sample handling instructions ➤ The kit should be stored at $2-8^{\circ}\text{C}$ and brought to room temperature for at least 30 minutes before use. ➤ Rat Alpha GST and RPA1 kit calibrator and positive control is stored at -20°C prior to use ➤ Samples should be brought to room temperature before commencing specimen preparation.
Check list for starting the assay	<ul style="list-style-type: none"> ➤ Bring all kit components (including calibrators and controls) and samples to room temperature. ➤ Prepare the wash solution (1/20 dilution). Ensure wash crystals are dissolved. If the wash solution does not dissolve completely this can be achieved by heating at 37°C for 10 minutes with stirring ➤ Dilute samples as required per kit specific instructions for use. Positive control does <u>not</u> require dilution. ➤ Mix the samples (and calibrators) using a mini vortex instrument then proceed as indicated in the pack insert. ➤ Conjugate is diluted in wash solution as directed in the instructions for use (there is no dilution required for rat alpha GST, GST Yb1 and RPA-1 conjugates EIAs). ➤ Prepare the calibrators according to the product insert. Calibrators must be used with 30 mins of preparation.
Points to note in the assay procedure	<ul style="list-style-type: none"> ➤ <u>Pipettes</u> should be <u>calibrated</u> regularly ➤ The <u>kit control</u> (PC) must always be included in duplicate to ensure assay validity ➤ All samples should be tested in <u>duplicate</u>. ➤ Where possible incubate the assay in a room temperature incubator. The assay performs optimally at $20-25^{\circ}\text{C}$. ➤ Incubations should be as indicated in the pack insert- the <u>substrate incubation</u> should be timed at <u>precisely 15 minutes</u>. ➤ Each calibrator dilution must be included to achieve an accurate standard curve.
Procedures to follow when handling reagents	<ul style="list-style-type: none"> ➤ Take particular care not to contaminate the <u>conjugate</u> with a used tip or other laboratory utensils. ➤ Wear gloves and suitable protective clothing when handling samples and kit reagents. ➤ Change gloves regularly, particularly when handling the substrate solution. ➤ Always change the pipette tips. ➤ Pipette the required amount of reagent into a <u>clean or new trough</u>.

	Possible cause	Solution
High Absorbance (high optical densities)	1. Cross contamination from other specimens	Repeat, taking care when washing and pipetting.
	2. Insufficient washing	Check washer is functioning correctly. Any minor deviations from the product insert regarding washing could lead to high background. Strips need to be washed 4 times with 250-350µL of wash solution after the sample and conjugate steps. Ensure all wash buffer is removed from wells after washing. NB. Only use Argutus kit wash solution.
	3. Salt crystals remaining in the wash concentrate	Salt crystals must be fully dissolved before the wash buffer is made up by gently heating the wash to 37°C for ~30 mins. Once crystals are dissolved, ensure wash concentrate is diluted as stated in the insert.
	4. Conjugate preparation.	The conjugate concentrate is dissolved 1/51 in wash solution . Instructions in diluting the conjugate must be followed carefully per microassay strip – see insert. Use immediately after preparation.
	5. Wavelength or filter not correct	Check that the wavelength is set at 450nm. Check calibration of instrument. Ensure that the reader has been serviced in line with manufacturers recommendations
	6. Assay background, false positive results for specimen	Check washer is functioning correctly as incorrect washing can lead to false positives. Ensure that the microtitre plate washer is decontaminated correctly (appendix I)
	7. Contaminated TMB (will have a blue colour)	Ensure TMB is colourless.
	8. Shaking or vibration of the assay plate during the TMB incubation step.	All steps in these assays require shaking of the plate <u>except</u> for the TMB incubation. Ensure no vibrations/shaking of the plate occurs during this step.
	9. Incorrect reagents used	Always use kit/lot specific reagents. Components from different batches of Argutus kits must not be intermixed.
	10. Incorrect incubation temperature, too high	Check that the room temperature incubator is set at the correct temperature. If an incubator is not being used, the temperature should stay within the recommended 20 -25°C.
	11. Incorrect incubation time, too long	Incubation times must be strictly adhered to, particularly the 15-minute TMB substrate incubation.
Low absorbance	1. Was the assay procedure followed correctly?	Have all the assay steps been carried out in accordance with insert?
	2. Incorrect preparation of reagents	Ensure the calibrators are made up as indicated in the product insert. The zero Calibrator is wash solution.
	3. Incorrect incubation temperature, too low	Check that the room temperature incubator is set at the correct temperature. If an incubator is not being used, the temperature should stay within the recommended 20 -25°C.
	4. Incorrect incubation time, too short	Incubation times must be strictly adhered to, particularly the substrate incubation.
	5. Kit has expired	Always check the expiry date, do not use if the kit has expired.
	6. Incorrect storage of kit	Kits must be stored at 2-8°C. Rat Alpha GST and RPA-1 EIA Calibrator and Positive control must be stored at -20°C
	7. Kit reagents not at room temperature	Allow sufficient time for the kit reagents to reach room temperature before starting the assay (~1hr).
	8. Incorrect reagents used	Assay components are kit and batch specific. Do not mix reagents from different batches.
Argu	9. Over washing of plate (e.g. inclusion of a long soak step)	Repeat the assay following the procedure outlined in the 2 pack insert.

Trouble Shooting Guide for the Collagen IV EIAs

BIO83 – Urinary Collagen IV

BIO82 – Serum Colalgen IV

Problem	Checks
Low optical density	<ul style="list-style-type: none"> • Check reader wave length • Check incubation time and temperature • Reagents not equilibrated to RT before use • Check preparation of reagents • Improper storage of kit reagents
High optical density High zero standard value	<ul style="list-style-type: none"> • Ensure that every well is completely filled and emptied at every wash step • Ensure that automatic washers are functioning correctly • Blot plates on paper towel after washing • Check incubation time and temperature • Check preparation of reagents
Flat curve/poor reproducibility	<ul style="list-style-type: none"> • Check pipette calibration • Check preparation of working standards • Ensure that troughs used with multichannel pipettes are separated and dedicated to individual components • Insufficient washing procedure

DECONTAMINATION Procedure (intended for Biotek washers)

- At the end of the day prepare a four-liter of 1% Tergazyme solution (Disinfectant) and deionized water (Rinse).
- Fill a Bottle C with Tergazyme solution. Fill a Bottle A with four liters of deionized water.
- Run the **Decontamination** program and three times **RINSE** program.

DECONTAMINATION and **RINSE** programs details as follows:

- Full head washer (Biotek ELx405)

Program name	DECONTAMINATION (<i>manufacturer program</i>)	DAY_RINSE (<i>manufacturer program</i>)
Prime volume	400ml	400ml
Prime flow rate	7	7
Soak after prime	YES	NO
Soak duration	20 min.	-
Overall time	app. 21min.	app. 1 min.

- Single head washer (Biotek ELx50)

Program name	DECONTAMINATION (<i>manufacturer program</i>)	DAY_RINSE (<i>manufacturer program</i>)
Prime volume	60ml	60ml
Prime flow rate	6	6
Soak after prime	YES	NO
Soak duration	20 min.	-
Overall time	app. 21min.	app. 1 min.

TERGAZYME information

Tergazyme Enzyme Active Powdered Detergent is a concentrated, anionic detergent with protease enzyme for manual and ultrasonic cleaning. Tergazyme is authorized by USDA for use in federally inspected meat and poultry plants. It passed inhibitory residue test for water analysis and is FDA certified.

a) *Ingredients*

C.A.S.	CONCENTRATION (%)	Ingredient Name
25155-30-0	10-30	Sodium Dodecylbenzenesulfonate
497-19-8	7-13	Sodium Carbonate
7758-29-4	30-40	Sodium Phosphate

b) Distributors (Republic of Ireland & United Kingdom)

IIS Ltd Phone: +35312867777
Unit 1 & 2, Ballywaltrin Business Park Fax: +35312867766
Boghall Road Email: CDrysdale@isisco.ie
Bray, Co. Wicklow Web Site: www.isisco.ie
Republic of Ireland

PC International Limited Phone: +44-1223893402
33 Westfield Fax: +44-1223894257
Abington, Cambridge CB1 6BE Email: pcinternational@btinternet.com
United Kingdom

c) Directions

Make a fresh 1% solution (e.g. 10 grams per liter) in cold or warm water. If available, use warm water below 55° C. For critical cleaning, do final or all rinsing in distilled, deionized, or purified water.

d) Other details

Manufacturer product code: 1304 (1.8kg)
Price: 36.60 Euro (IIS Ltd)
1800g Tergazyme (1 box) = 180 Liters of 1% solution