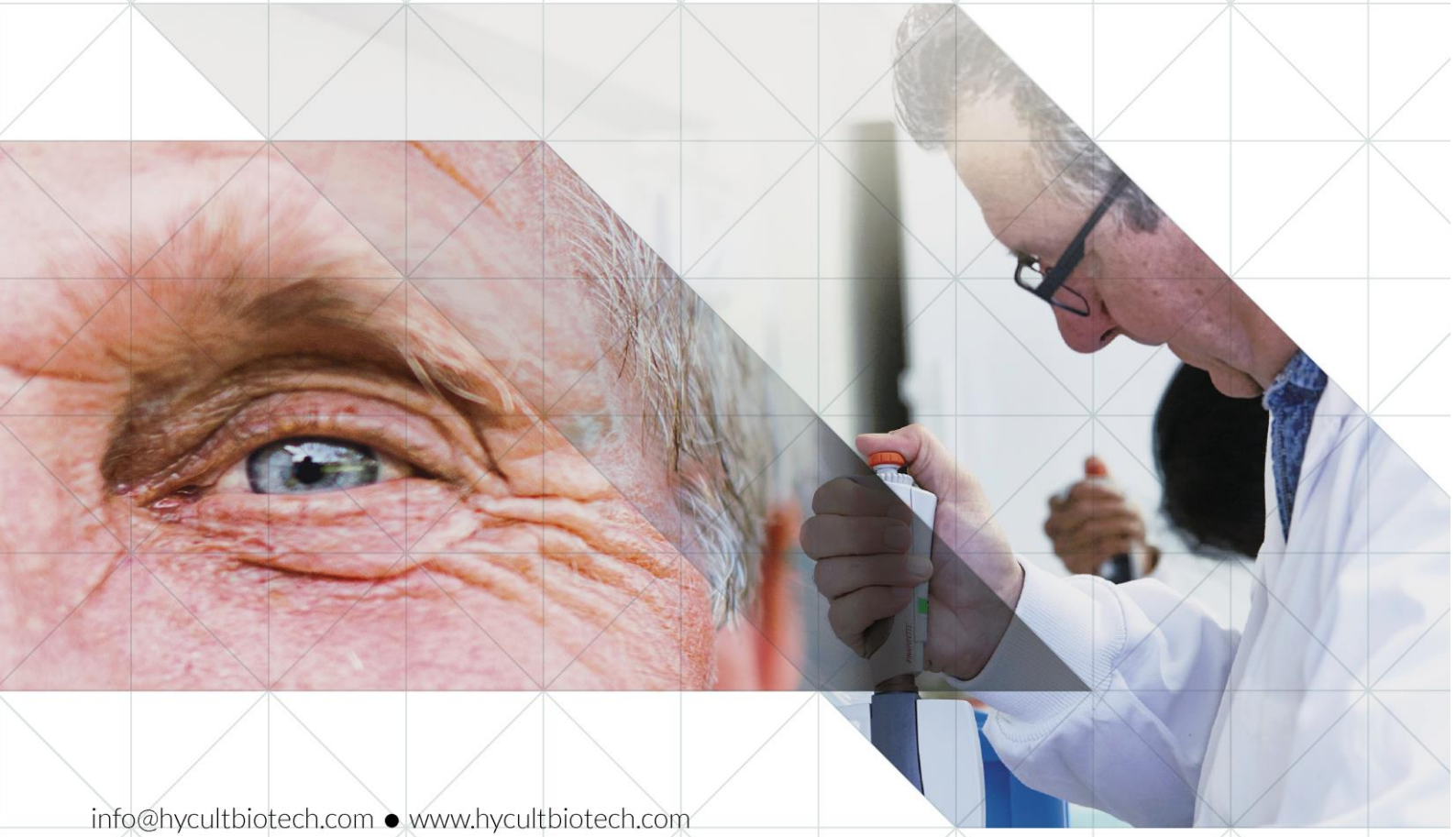
Arginase Activity Assay

HIT505

Edition 12-19

ASSAY
PRODUCT INFORMATION & MANUAL

Read carefully prior to starting procedures!
For use in laboratory research only
Not for clinical or diagnostic use



info@hycultbiotech.com ● www.hycultbiotech.com

Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the Certificate of Analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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1. INTENDED USE

The Arginase Activity Assay kit is to be used for *in vitro* quantitative determination of active arginase in tissue lysate samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

The analysis should be performed by trained laboratory professionals.

2. INTRODUCTION

The Arginase activity assay is a ready-to-use assay for the quantification of the enzymatic activity of Arginase in tissues samples. Arginase is an hydrolytic enzyme that converts arginine to ornithine and urea (fig 1). It is ubiquitous to all domains of life. The enzyme exists as two isoforms, arginase-I and arginase-II, which are encoded by different genes. Although arginase-I and arginase-II have similar enzymatic activities, they differ in isoelectric point (pI) and immunological reactivity. Human arginase-I (or liver-type arginase) is a 35 kD protein circulating in blood probably as a homotrimer. Liver-type arginase functions mainly in the urea cycle and is therefore abundantly expressed in mammalian liver. Arginase II is expressed in mitochondria of several tissues in the body, such as the kidney and prostate. It may also be found at lower levels in macrophages, lactating mammary glands, and brain tissue. In addition to its involvement in ammonia detoxification via the urea cycle, arginase plays a role in other processes, for instance macrophage-mediated cytotoxicity due to arginase release and inhibition of lymphocyte proliferation in vitro. It shows high activity in growing tissues, wound healing, proliferating lymphocytes and tumors. Furthermore, arginase acts as a modulator of the immune response. Besides this, arginase plays a role in allergen challenged lungs, in autoimmune inflammation in the central nervous system and in acute liver injury.

The Arginase activity assay provides an easy and fast procedure for measuring arginase activity in several sample preparations such as tissue- or cell lysates. Arginine solution (included as component is this kit) is incubated together with the samples of interest. The arginase present in these samples will convert arginine to urea and ornithine. The produced urea will react with the substrate ISPF (α-Isonitrosopropiophenone) thereby generating a colored product that can be measured and quantified. The assay has a detection limit of 0.35 units per mg protein.

3. KIT FEATURES

- Working time of 1 hour and 40 minutes.
- Minimum activity that can be measured in a 40x sample dilution is 0.35 Units/mg protein.

Cross-reactivity

The assay detects urea that is formed from arginine by active arginase. The assay can be used in all species in which this process occurs (fig. 1).

4. PROTOCOL OVERVIEW

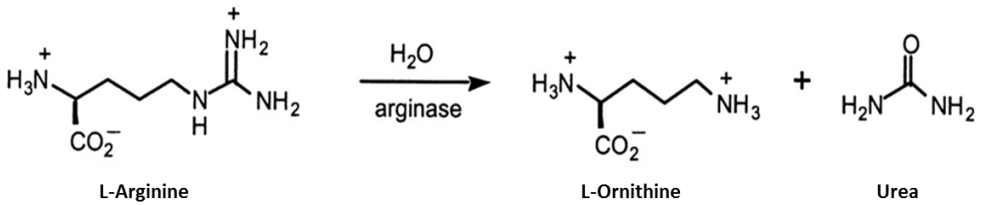
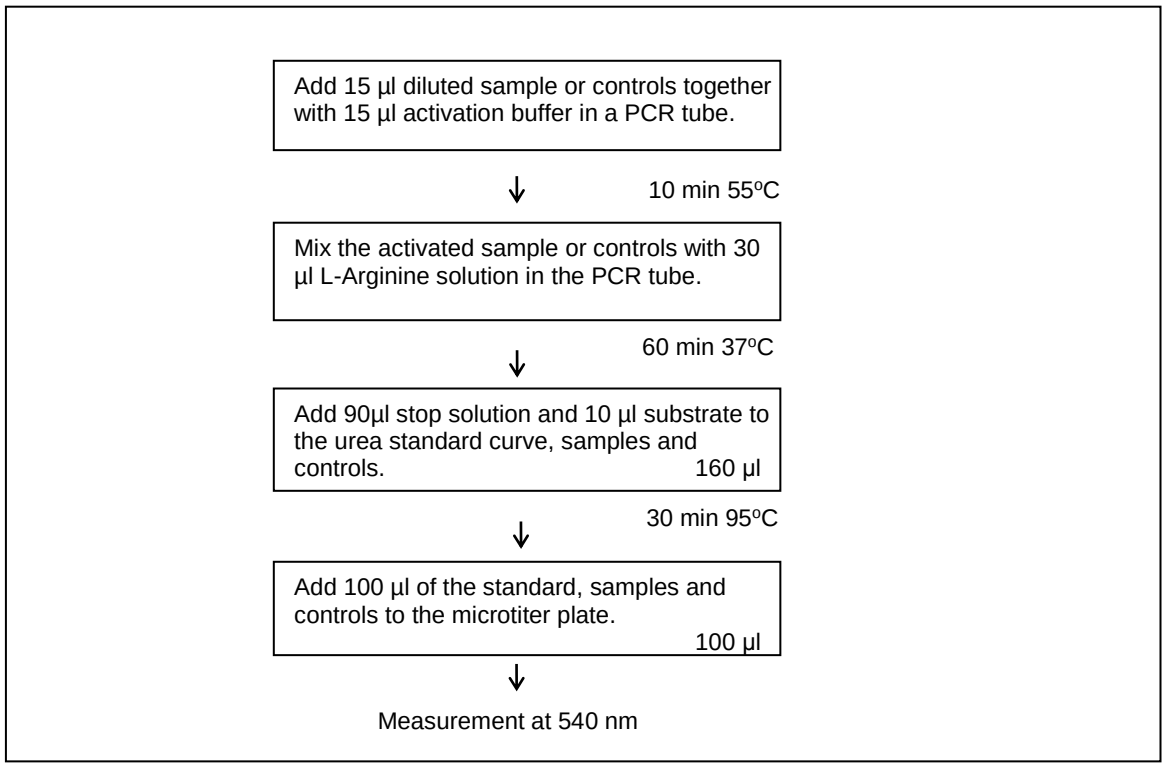


Fig.1: Conversion of L-Arginine to L-ornithine and Urea by Arginase and H_2O.



- The Arginase activity assay is a ready-to-use, fast assay based on the conversion of Arginine to Urea.
- The efficient format of a plate with twelve disposable 8-tube PCR strips and twelve disposable 8-well microtiter strips allows free choice of the number of samples that is analyzed in the assay.
- Samples and controls are activated with a manganese solution.
- Activated samples, are next to the controls, hydrolyzed with Arginine.
- Hydrolysis is stopped by adding stop solution.
- The substrate, α-isonitrosopropiophenone (ISPF) will react with the newly formed urea, resulting in a color change which can be measured.
- The absorbance at 540 nm is measured with a spectrophotometer. A urea curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of urea.
- The arginase activity of the samples and controls, can be determined using the urea curve and provided formula.

5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Cat.#	Quantity	Color code
Sample activation buffer A 5x	SB001	1 vial (1 ml)	White
Sample activation buffer B 2x	SB003	1 vial (2.5 ml)	Colorless
Arginine solution	SB002	1 vial (3 ml)	Blue
Dilution buffer	DB106	1 vial (60 ml)	Green
Standard		2 tubes, in solution	Red
Negative control		2 vials, lyophilized	Blue
Positive control		2 vials, lyophilized	Red
ISPF substrate	SUB001	1 tube, 1 ml in solution	Brown
Stop solution	STOP113	1 vial (11 ml)	Red
12 PCR-strips (8-tube)	HIT505-PCR	12 strips	
Microtiter plate	HIT505-P	1 plate	
Certificate of Analysis		1	
Manual		1	
Data collection sheet		2	

Table 1

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- All components in solution, the standard in concentrated solution and the controls in lyophilized form are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The controls are single use. After reconstitution the controls cannot be stored for repeated use.
- The controls should be interpreted as technical control in order to see whether assay performance fits the requirements. It is recommended to include your own reference samples.
- Upon receipt, foil pouch around the plates should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence assay performance.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed for 1 month if stored at 2 - 8°C.

Materials required but not provided

- Calibrated micropipettes and disposable tips.
- Polypropylene tubes
- Calibrated PCR machine.
- Calibrated ELISA plate reader capable of measuring absorbance at 540 nm.
- PCR with heated lid.

6. WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not add under any circumstances sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
- The assay has been optimized for the indicated range. Do not change the range.
- Open vials carefully: vials can be under vacuum.
- It is advised to spin down tubes before use.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell seek medical advice immediately. If in eyes rinse with water and seek medical advice immediately.
- The sample activation buffer contains manganese. Manganese is harmful in contact with skin or eyes and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The ISPF substrate is light sensitive, keep away from bright light. The solution should be colorless until use. The solution is prepared with 100% ethanol. 100% ethanol is highly flammable liquid and may cause damage to organs. In case of accident or if you feel unwell, seek medical advice immediately.
- The stop solution contains 10% H_2SO_4 and 30% H_3PO_4 and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse PCR tubes and/or microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.

7. SAMPLE PREPARATION

Collection and handling

Lysates

Lyse tissue samples on ice in lysis buffer: for instance 50mM TrisHCL pH7.4, 1% NP-40, 0.25% Na-deoxycholate, 150mM NaCl and 1mM EDTA. Add 200 µl lysis buffer to 10 mg tissue before homogenization. Homogenize on ice using standard methods to open cell membranes, for example by Potter-Elvehjem, blender or sonicator technique. Centrifuge samples (maximum speed at 4°C for 30 min) to avoid contamination with cellular debris.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C may affect the arginase activity. Avoid multiple freeze-thaw cycles which may give erroneous results. Samples should be thawed on ice. Prepare all samples prior to starting the assay procedure.

Dilution procedures

Lysates

This assay is optimized for a 40x dilution of a 1 mg/ml tissue lysate. If the 40x dilution is out of range, it is advised to re-analyse the sample using a higher dilution.

Remark regarding recommended sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples

Please see table 2 for recommended sample dilution, which is sufficient for one sample in duplicate in the activity assay.

	Dilution	Pre-dilution	Amount of sample or pre-dilution required	Amount of Dilution buffer required
1.	40x	1 mg/ml concentration required	5 µl (sample)	195 µl

Table 2

8. REAGENT PREPARATION

Allow all the reagents to adjust to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Sample activation buffer A and B

Prepare sample activation buffer by mixing 600 µl of the 5x sample activation buffer A with 1500 µl of sample activation buffer B and 900 µl distilled or de-ionized water.

This 3 ml is sufficient for 1 x 96 tests. Where less volume is required, prepare the desired volume of sample activation buffer by diluting 1 part sample activation buffer A with 2.5 parts sample activation buffer B and 1.5 parts distilled or de-ionized water.

Standard

Prepare each standard in polypropylene tubes by serial dilution of the standard with dilution buffer as shown in Figure 2*.

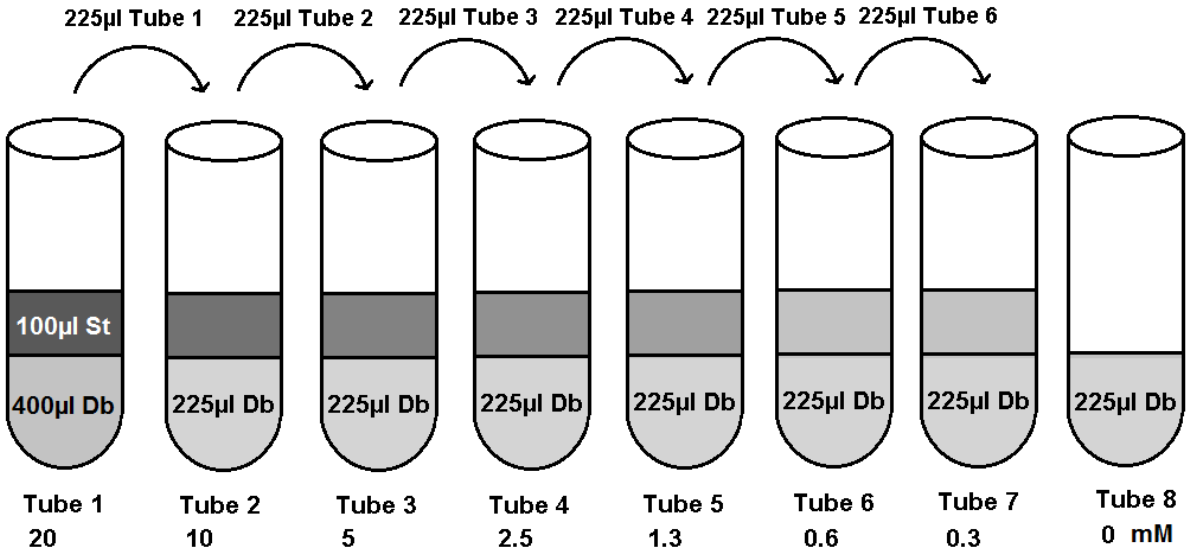


Figure 2

*) CoA: Certificate of Analysis, St: Standard, Db: Dilution buffer.

Positive and Negative control

The controls are reconstituted by pipetting 0.25 ml dilution buffer. After reconstitution, the controls are ready to use.

9. PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine number of test wells / tubes required and return the unused strips to the storage bag with desiccant, seal and store at 2-8°C.
2. Bring all lysated samples to a protein concentration of 1 mg/ml.
3. Subsequently, dilute samples 40x.
4. Add 15 µl sample or 15 µl control together with 15 µl activation buffer to a PCR-tube.
5. Incubate the samples and controls for 10 minutes at 55°C*.
6. Mix the activated samples and controls with 30 µl of L-Arginine solution in the PCR tube. Incubate the samples for 60 minutes at 37°C.
7. Prepare the Urea standard curve as indicated and add 60 µl of each standard concentration to a PCR tube.
8. Add 90 µl stop solution and 10 µl substrate to the standard, controls and samples. Incubate standard, controls and samples for 30 minutes at 95°C.
9. Transfer 100 µl standard, controls and sample to the microtiter plate.
10. Read the plate at 540 nm using a plate reader, following the instructions provided by the instruments manufacturer.

* This assay has been optimized with a PCR machine. Heating with other equipment (e.g. a water bath) is possible, however, this has not been tested.

10. INTERPRETATION OF RESULTS

- The mean absorbance of the zero standard should be less than 0.2.
- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
- Calculate the total amount of formed urea of all samples using the standard curve.
- Calculate arginase activity : Arginase activity is represented as units/mg protein and can be determined using the formula:
    - Arginase activity: total amount urea formed * 1,11 = unit/mg protein
        - Where: activity calculated from curve is mM Urea formed in 1 h.
        - 1,11: mg protein in minutes conversion factor
        - 1 Unit = the amount of enzyme that will generate 1 mM Urea per minute.
- Positive control sample should have activity > 6 Units/ mg protein
- Negative control sample should have activity <1 Unit/mg protein

11. TECHNICAL HINTS

- User should be trained and familiar with activity assays and test procedure.
- If you are not familiar with the technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Samples should be referred to the standard curve prepared on the same plate.

- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
- Each time the kit is used fresh dilutions of all components should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- The waste disposal should be performed according to your laboratory regulations.

Technical support

Do not hesitate to contact our technical support team at support@hycultbiotech.com for inquiries and technical support regarding the Arginase Activity Assay.

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12. QUALITY CONTROL

The Certificate of Analysis included in this assay is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the Certificate of Analysis are to be used as a guideline only. The results obtained by your laboratory may differ. For optimal performance of this assay, it is advised to work according to good laboratory practice.

13. TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data shall be sent to support@hycultbiotech.com.

Suggestions summarized below in Table 3 can be used as guideline in case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•	•	Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•	•	•	•	•	Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
•		•			Especially in case of 37 or 95°C incubation: plates are not incubated uniformly
•					Assay performed before reagents were adapted to room temperature
•	•	•	•	•	Procedure not followed correctly
			•	•	Omission of a reagent or a step
		•			Poor mixing of samples
•	•	•			Cross-contamination from other samples or positive control
		•	•		ISPF substrate is not clear or colorless
•	•				Wrong filter in the microtiter reader
	•	•			Airbubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions
•					Lamp in microplate reader is not functioning optimally

Table 3