PATHOZYME® ElisaSure Ref OD707

For the routine testing of performance of microplate washers, microplate readers, pipettes and pipetting devices.

Store at 2°C to 8°C. DO NOT FREEZE

INTRODUCTION

Instruments and Equipment are an essential part of Microplate based test procedures. Their performance must be reproducible within known parameters between maintenance periods and after repair or irregular results by easy and convenient methods.
The **Pathozyme ElisaSure** kit provides the ability to test all Microtitre Plate Washers, Microplate Readers, manual and automated pipetting devices.
Regular use of the **Pathozyme ElisaSure** test kit will produce documented records evidencing equipment performance and will assist in the early detection of deficiencies and assist in troubleshooting by incorporating four separate equipment checks.
Regular monitoring will, in the event of encountering unexpected results during sample testing, provide a means of comparison between current and historical performance and, by incorporation into the laboratories maintenance schedule, provide confidence in the performance of equipment.
For professional use only.

INTENDED USE

Pathozyme ElisaSure provides the means to check the linearity and precision of Microplate Readers, the efficiency and reproducibility of Microplate Washers and the precision of manual and automated Pipettes.

PRINCIPLE OF THE TEST

Microplate Reader Linearity
Dilutions of coloured solution are read at the appropriate wavelength: 405, 450 or 492 nm.
The readers range and linearilty are established.

Microplate Reader Precision and Microplate Washer test.
A solution containg HRP is added to microwells and read at 405, 450 or 492 nm. The microwells are washed. On addition of the Substrate (TMB), a colour will develop only in those wells in which HRP is present. The reaction is stopped by the addition of dilute Sulphuric Acid and the absorbance is then measured at 405, 450 or 492 nm. The level of HRP remaining is directly proportional to the intensity of colour development and the efficiency of the Microtitre Plate Washer.

Pipetting Device test.
Duplicate dilutions of dye are prepared and transferred to Microtitre Plate wells and read at the appropriate wavelength: 405, 450 or 492 nm.
The degree of difference in Mean OD and CV% levels are indicators of the reproducibility of the volume dispensed.

There are no International standards for this test.

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Microtitre Plate	**12 x 8 wells x 1**
Breakable wells.	
Dye **Conc**	**1ml**
Dye concentrate with protein stabilisers.(Red)	
Conj	**15 ml**
Anti-human IgG Conjugate: Anti-human IgG conjugated to Horseradish Peroxidase. Ready to use. (Purple)	
Washbuf **20X**	**50 ml**
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)	
Subs **TMB**	**15 ml**
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	
Soln **Stop** **H2SO4** **0.2M**	**15 ml**
Stop Solution: Sulphuric Acid diluted in purified water. Ready to use. (Colourless)	
Instruction Leaflet and Lin / Log graph paper	**1 + 1**

MATERIAL REQUIRED BUT NOT PROVIDED

Test Tubes
Microplate reader
Microplate washer
Pipetting Device
Thoroughly clean laboratory glassware.
Purified Water

PRECAUTIONS

Pathozyme ElisaSure reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

Pathozyme ElisaSure Stop Solution is diluted Sulphuric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

Pathozyme ElisaSure reagents contain 1% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

*Proclin™ 300 is a trade mark of ROHM & HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

Wash Buffer:
Dilute the concentrated Wash Buffer using 1 part Wash Buffer concentrate with 19 parts distilled water. For every 8-well breakable strip, prepare 25ml of diluted Wash Buffer by adding 1.25ml of concentrated Wash Buffer to 23.75ml of distilled water. Prepare fresh diluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable priming of automatic washing machines.

LIMITATIONS OF USE

Levels stated are provided only as a guide. For levels specific to an individual article of equipment or setting please consult the manufacturer's Instruction Manual.

This product is not a replacement for Instrument Calibration.

There is no reuse protocol for this product.

RECOMMENDED TESTING REGIME

	TEST		
	LINEARITY	**PRECISION / WASHER**	**PIPETTORS**
PERIOD	**Once a Month**	**Once a Week**	**Once every 2 Weeks (bi-weekly)**
MATERIALS		**2 Strips**	
			1 Strip
	2 Strips		
TOTAL	**2 Strips / Month**	**8 Strips / Month**	**2 Strips / Month**

MICROPLATE READER LINEARITY

ASSAY PROCEDURE

Recommended to be performed every month, in the event of encountering unexpected results during sample testing or after repair or maintainance.

1. Bring all the kit components to room temperature (20°C to 25°C) prior to the start of the assay.
2. Prepare a 1/10 Dye solution by adding 100µl of Dye Conc to 900 µl Purified Water and mix well.
3. Secure one strip of wells in the holder.
4. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

With reference to Table below:

5. Add 100µl of Purified Water to Well A and Wells C to H inclusive.
6. Add 100µl of 1/10 Dye solution to Well B and Well C.
7. Double dilute 100µl volumes from Well C to Well H.
8. Mix well between each transfer.
9. Discard 100µl volume remaining in pipette after Well H.
10. Zero the microplate reader using Well A.
11. Measure the Optical Density (OD) at 405, 450 or 492 nm as appropriate.

Well	Dye Dilution	
	Replicate 1	Replicate 2
	Column 1	Column 2
A	Blank (No Dye)	Blank (No Dye)
B	1/10	1/10
C	1/20	1/20
D	1/40	1/40
E	1/80	1/80
F	1/160	1/160
G	1/320	1/320
H	1/640	1/640

CALCULATION OF RESULTS AND EXPECTED VALUES

Plot OD versus dilution on Lin / Log graph paper.
Readers should be Linear to OD 1.0 (consult Manufacturers Instruction Manual).
Data gained should be referenced against previous figures to monitor Instrument function.
Reproducability between duplicate strips confirms pipette precision and method.
Ref Figure 1 overleaf.

MICROPLATE READER PRECISION AND MICROPLATE WASHER TEST

ASSAY PROCEDURE

Recommended to be performed every week, in the event of encountering unexpected results during sample testing or after repair or maintainance.

1. Bring all the kit components to room temperature (20°C to 25°C) prior to the start of the assay.
2. Secure two strips of wells in the holder.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

With reference to Table below:

4. Add 100µl of Conjugate to each well.
5. Zero the microplate reader against air.
6. Measure the OD at 405, 450 or 492 nm as appropriate **These readings are used to calculate microplate reader precision.**
7. Wash plate four times with wash buffer with out tapping out. (Reducing the number of washes will increase the sensitivity of the test – increasing the number of washes will decrease the sensitivity of the test).
8. Add 100µl of Substrate Solution to each well.
9. Incubate in the dark at room temperature (20ºC to 30ºC) for 10 minutes.
10. Add 100µl of Stop Solution to each well.
11. Zero the microplate reader against air.
12. Measure the OD of each well at 405, 450 or 492 nm as appropriate IMMEDIATELY. **These readings are used to calculate the microplate washer efficiency.**
13. If a reference filter between 600 and 650 nm is used readings will appear artificially lower.

Row	Column 1	Column 2
A		
B		
C		
D		
E		
F		
G		
H		

CALCULATION OF RESULTS AND EXPECTED VALUES

MICROPLATE READER PRECISION

1. Calculate the Mean OD from the readings obtained from point 6 above.
2. The Mean value for ensuing assays should not differ by >10%.
3. Calculate the Standard Deviation, divide by the Mean and multiply the result by 100. The resultant figure is the Coefficient of Variation (CV%)
4. CV's > 5% should be further investigated. **Ref Figure 2.**

MICROPLATE WASHER TEST

1. Use Absorbance readings obtained from point 12 above.
2. Individual readings > 0.10 indicate inefficient washing.
3. If readings are above this limit the test should be repeated and the equipment manufacturers trouble shooting procedures followed. **Ref Figure 3.**

PIPETTING DEVICE TEST

ASSAY PROCEDURE

Recommended to be performed bi-weekly, in the event of encountering unexpected results during sample testing or after repair or maintainance.

Manual Pipetting:

1. Bring all the kit components to room temperature (20°C to 25°C) prior to the start of the assay.
2. Prepare a 1/10 Dye solution by adding 10µl of Dye Conc to 90µl Purified Water and mix well.
3. Secure a minimum of eight wells in the holder.
4. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
5. Add 10µl of 1/10 Dye solution to each well.
6. Add 90µl of Purified Water to each well.
7. Zero the microplate reader against air.
8. Measure the absorbance at 405, 450 or 492 nm as appropriate.

Automated Pipetting:

1. Bring all the kit components to room temperature (20°C to 25°C) prior to the start of the assay.
2. Submit eight vials each containing a sample of neat Dye Conc solution.
3. Secure a minimum of eight wells in the holder.
4. Instruct the automatic pipette to prepare 1/100 dilutions using Purified Water.
5. Zero the microplate reader against air.
6. Measure the absorbance at 405, 450 or 492 nm as appropriate.

CALCULATION OF RESULTS

1. Calculate the Mean OD's for points 8 and 6 above..
2. For both sets of figures calculate the Standard Deviation, divide by the Mean and multiply the result by 100.
   a. The resultant figures are the Coefficients of Variation (CV%).
   b. Both figures should be less than 5%.
   c. The difference between both figures should be within 5%.
3. Data gained should be compared with previous figures to monitor variation over time.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or strips from different kits.

Do not allow reagent to run down the sides of the well.

Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

For technical advice concerning specific settings, trouble shooting, calibration or repair relating to an individual article of equipment please consult the manufacturer's Instruction Manual.

REFERENCES

(1) **Wild, D. (1994).** The Immunoassay Handbook. Stockton Press, New York.

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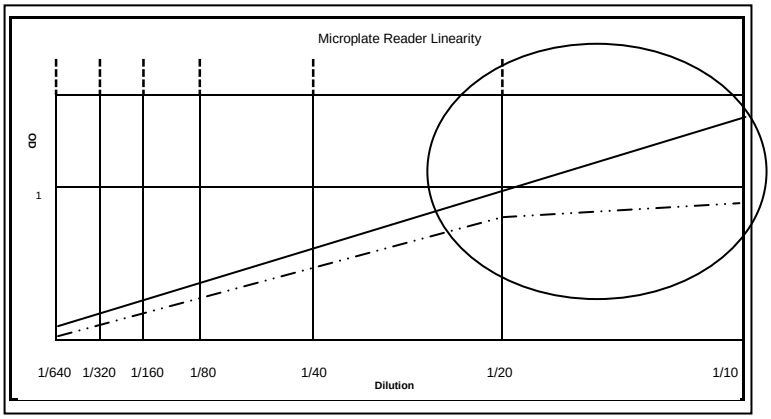
Figure 1
Microplate Reader Linearity EXAMPLE

Reader A
Reader B



Results:
Reader A - Linear to OD 1.0
Reader B - Not Linear to OD 1.0

Figure 2
Microplate Reader Precision (example figures)

	OD			
Row	Reader A		Reader B	
A	0.431	0.417	0.430	0.424
B	0.435	0.427	0.613	0.625
C	0.425	0.420	0.620	0.613
D	0.418	0.421	0.426	0.419
E	0.429	0.418	0.419	0.416
F	0.415	0.422	0.422	0.429
G	0.414	0.430	0.418	0.412
H	0.425	0.433	0.427	0.417

Example figure interpretation:
Reader A Acceptable – Mean 0.423 SD 0.00639 CV% 1.51
Reader B Not Acceptable – Mean 0.470 SD 0.0851 CV% 18.11 (Greater than 5%)
Note High readings in Rows B and C – Investigation required.

Figure 3
Microplate Washer Test (example figures)

	OD			
Row	Washer A		Washer B	
A	0.045	0.042	0.043	0.039
B	0.042	0.040	0.041	0.042
C	0.047	0.040	0.041	0.045
D	0.045	0.043	0.145	0.139
E	0.044	0.045	0.140	0.142
F	0.042	0.041	0.042	0.039
G	0.044	0.047	0.039	0.040
H	0.047	0.044	0.044	0.043

Example figure interpretation:
Washer A Acceptable –
Washer B Not Acceptable – Note High readings in Rows D and E – Investigation required.

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