


# EIAgen Combi 6 KIT

[REF] 11C6-100C

 96 Tests

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**FOR IN VITRO DIAGNOSTIC USE ONLY**

Store at 2...8 °C




**en**  
**SYMBOLS USED ON LABELS**

[IVD]	In vitro diagnostic medical device (In vitro diagnostic use)
[LOT]	Lot number
[REF]	Catalogue Code
[MT_PLATE]	Microplate
[CONJ HRP]	Conjugate
[CAL__SSA]	Calibrator SSA
[CAL__SSB]	Calibrator SSB
[CAL__Sm]	Calibrator Sm
[CAL__Sm/RNP]	Calibrator Sm/RNP
[CAL__Jo-1]	Calibrator Jo-1
[CAL__Scl-70]	Calibrator Scl-70
[CONTROL__SSA +]	SSA Positive Control
[CONTROL__SSB +]	SSB Positive Control
[CONTROL__Sm +]	Sm Positive Control
[CONTROL__Sm/RNP +]	Sm/RNP Positive Control
[CONTROL__Jo-1 +]	Jo-1 Positive Control
[CONTROL__Scl-70 +]	Scl-70 Positive Control
[CONTROL -]	Negative Control
[SOLN TMB]	Substrate (TMB)
[DILSPE]	Sample Diluent
[BUF WASH 20x]	Washing Buffer 20X
[SOLN STOP]	Stop Solution

 Expiry date (Use by...)

 8°C  
2°C Temperature limitation (store at 2...8°C)

 Number of tests


Keep away from sunlight

**M**

Manufactured by



Attention, See Instructions For Use



Biological risk

## 1.0 INTENDED USE

The EIAgen Combi 6 is an enzyme-linked immunosorbent assay method for the simultaneous semi-quantitative determination of specific IgG autoantibodies to SS-A/Ro, SS-B/La, Sm, Sm/RNP, Jo-1 and Scl-70 in human serum. The results of the Combi 6 assays can be used as an aid in the diagnosis of auto-immune diseases including Systemic Lupus Erythematosus (SLE), Sjögren's Syndrome (SS), Mixed Connective Tissue Disease (MCTD), Poly/Dermato myositis (PM/DM) and Scleroderma. Levels of these autoantibodies are one indicator in a multi-factorial diagnostic regime.

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

The detection of Anti-Nuclear Antibodies (ANA) has long been an important tool in the diagnosis of systemic rheumatic diseases. The antigens used in their detection are purified by the saline extraction of human or animal nuclei, this has led to them being termed Extractable Nuclear Antigens (ENA). The most commonly measured ENA specifications are anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-Sm/RNP, anti-Jo-1 and anti-Scl-70.

The intracellular antigens SS-A/Ro, SS-B/La, Sm, Sm/RNP, Jo-1 and Scl-70 are targets for autoimmune responses in many patients with rheumatic diseases. Anti-SS-A/Ro antibodies are found in 30-50% of patients with Systemic Lupus Erythematosus (SLE), but most significantly in around 95% of patients with primary or secondary Sjögren's Syndrome (SS). Anti-SS-B/La antibodies are also found in SLE and SS patients. Anti-Sm antibodies are considered highly specific for SLE and approximately 30-40% of patients show their presence. Anti-RNP antibodies are predominantly found in patients with Mixed Connective Tissue Disease (MCTD) but are also associated with SLE, SS and Scleroderma. Anti-Jo-1 antibodies are considered specific for Polymyositis and Dermatomyositis. Anti-Scl-70 antibodies are recognised as specific markers for Primary Systemic Sclerosis (PSS or Scleroderma).

Autoantibodies to ENA's vary depending on disease state. The following are estimated incidences of ENA antibodies in various diseases:

Antibody	SLE	SS	PSS	MCTD	PM	DM
SS-A/Ro	30-50%	~95%	---	---	---	---
SS-B/La	>15%	~87%	---	---	---	---
Sm	30-40%	---	---	---	---	---
Sm/RNP	35-45%	>30%	>20%	95-100%	---	---
Jo-1	---	---	---	---	>25%	>25%
Scl-70	---	---	20-30%	---	---	---

## 3.0 PRINCIPLE OF THE ASSAY

The EIAgen Combi 6 assay for detection of autoantibodies is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a colour reaction of an enzyme and substrate. The EIAgen wells are coated with purified antigens. The microplate supplied contains two strips coated with each of the individual antigens. On adding diluted serum to the wells the antibodies present bind to the antigen. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG monoclonal antibody (HRP Anti-IgG) is added, which binds to the immobilised antibodies.

Following further incubation and washing, tetra-methyl benzidine substrate (TMB) is added to each well. The presence of the antigen-antibody-conjugate complex turns the substrate to a dark blue colour. Addition of the stop solution turns the colour to yellow.

The colour intensity is proportional to the amount of autoantibodies present in the original serum sample.

## 4.0 KIT COMPONENTS

### 4.1 MICROPLATE [MT\_PLATE]

(code 53PIA) One microplate is supplied which contains 16 wells of each antigen in a breakapart format. The wells are coated with purified ENAs of bovine origin, and are colour-coded according to the antigen:

<b>SS-A</b>	yellow	<b>Sm/RNP</b>	red
<b>SS-B</b>	light blue	<b>Jo-1</b>	white
<b>Sm</b>	green	<b>Scl-70</b>	dark blue

**4.2 CALIBRATORS** Six vials each containing 1ml of calibrator. Each calibrator represents 100u/ml for that parameter. The calibrators are in arbitrary units and contain human antisera and 0.09% sodium azide as a preservative.

Calibrator	symbol	code	Anti-ENA
SSA calibrator	[CAL__SSA]	53CALSSA	Anti-SSA/Ro
SSB calibrator	[CAL__SSB]	53CALSSB	Anti-SSB/La
Sm calibrator	[CAL__Sm]	53CALSM	Anti-Sm
Sm/RNP calibrator	[CAL__Sm/RNP]	53CALRNP	Anti-Sm/RNP
Jo-1 calibrator	[CAL__Jo-1]	53CALJO	Anti-Jo-1
Scl-70 calibrator	[CAL__Scl-70]	53CALSCL	Anti-Scl-70

**4.3 CONJUGATE** [CONJ|HRP]  
(code 53HRP) One vial containing 15ml of ready-to-use HRP conjugate. Conjugate contains 0.05% Proclin 300. Conjugates are color coded pink.

**4.4 POSITIVE CONTROLS** Six vials each containing 0.45 ml of concentrated positive control which contains human antisera and 0.09% sodium azide as a preservative. There is one positive control for each parameter.

Control	symbol	code	Anti-ENA
SSA positive control	[CONTROL__SSA +]	53POSSSA	Anti-SSA/Ro
SSB positive control	[CONTROL__SSB +]	53POSSSB	Anti-SSB/La
Sm positive control	[CONTROL__Sm +]	53POSSM	Anti-Sm
Sm/RNP positive control	[CONTROL__Sm/RNP +]	53POSSmRNP	Anti-Sm/RNP
Jo-1 positive control	[CONTROL__Jo-1 +]	53POSJO	Anti-Jo-1
Scl-70 positive control	[CONTROL__Scl-70 +]	53POSSCL	Anti-Scl-70

**4.5 NEGATIVE CONTROL** [CONTROL|-]  
(code 53NEG) One vial containing 0.45 ml of concentrated negative control which contains normal human serum and 0.09% sodium azide as a preservative. This can be used for each of the parameters.

The controls (positive and negative) are provided in a concentrated form and should be diluted 1/100 with sample diluent buffer before use. Prepare fresh control dilutions before each assay run. Vortex all samples and controls before testing.

**4.6 SUBSTRATE (TMB)** [SOLN|TMB]  
(code TMBC) One vial containing 15ml of ready-to-use tetra-methylbenzidine (TMB) substrate.

**4.7 SAMPLE DILUENT** [DILSPE]  
(code 53SD) Two bottles containing 60ml/each of ready-to-use sample diluent buffer. The buffer includes 0.09% Sodium azide. Sample diluent buffer is color coded blue.

**4.8 WASHING BUFFER** [BUF|WASH|20x]  
(code TLAVC) conc. 20X One bottle containing 50ml of wash buffer concentrate. Wash buffer concentrate contains 0.06% Proclin 300.  
Dilute the whole content of the bottle up to one liter with distilled or deionized water. Mix well before use. Store this solution at 2...8°C if it is not to be used immediately. The diluted wash buffer is stable at 2...8°C for one week.

**4.9 STOP SOLUTION** [SOLN|STOP]  
(code STOPC) One bottle containing 20ml of 0.25M H<sub>2</sub>SO<sub>4</sub> stop solution.  
**The MSDS is available upon request of laboratory personnel.**

**5.0 STORAGE AND STABILITY AFTER THE FIRST OPENING**  
-Store kit components at 2...8°C and do not use after the expiry date on the box outer label.  
-Before use all components should be allowed to warm up to ambient temperature (18...25°C).  
-After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2...8°C.  
-The opened kit should be used within three months.

**6.0 MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED**

- Distilled or deionized water.
- Wash bottle, automated or semi-automated microwell plate washing system.

- Rack for sample dilution.
- Micropipettes including multichannels capable of accurately delivering 10-1000µl (less than 3% cv).
- Reagent reservoirs for multichannel pipettes.
- One-liter graduated cylinder.
- Disposal basins and 0.5% sodium hypochlorite (50 ml bleach in 950ml water).
- Microtiter plate reader equipped for the measurement of the absorbance at 450 and 405 nm (reference filter at 620 nm).
- Paper towels, pipette tips and timer

## 7.0 WARNINGS AND PRECAUTIONS

### 7.1 SAFETY PRECAUTIONS

- All reagents in this kit are for in vitro diagnostic use only. Only experienced laboratory personnel should use this test and handling should be in agreement with GLP
- Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- Reagents contain preservatives which may be toxic if ingested. Do not pipette by mouth. Avoid contact of reagents or patient samples with skin or mucous membranes. If contact occurs, immediately flush with large quantities of water.
- Avoid splashing or creation of aerosols.
- Reusable glassware must be thoroughly washed and rinsed so that it is free of all detergents.
- Sera used in the preparation of the calibrators and controls have been tested for the presence of antibodies to Human Immunodeficiency Virus (HIV 1 and 2), as well as for Hepatitis B Surface Antigen (HBsAg) and HCV and found to be negative. All material is tested with FDA approved assays. Because no test method can offer complete assurance that HIV, HBsAg or other infectious agents are absent, it is recommended that human serum based products be handled with the same precautions used for patient specimens. Dispose of reagent solutions containing sodium azide and thimerosal as preservatives according to all local, state and national regulations. To dispose of reagents containing azide, flush away using copious amounts of water. Dispose with caution as azide can form explosive compounds on prolonged contact with lead or copper piping.

### 7.2 TECHNICAL PRECAUTIONS

#### A. Correct use of reagents and proper pipetting

- The performance data represented here were obtained using specific reagents listed in the package insert. Do not use reagents from other manufacturers in the kits.
- Do not use reagents from other EIAgen kits with this kit. Do not mix reagents from different kit lots.
- Do not dilute or adulterate the kit reagents, unless directed by the kit protocol. Do not use the substrate solution if it has begun to turn blue.
- Do not use heat-inactivated serum. Microplate washing is important. Improperly washed wells will give erroneous results.

#### B. Adherence to assay procedure and specifications

The obtained values have to be always compared to the ones reported in QC sheet. Do not use the kit to determine values outside the range indicated in the IFU. The test protocol must be followed strictly. Observe the indicated incubation times and temperature and the washing procedure, these are critical steps. Include the positive and negative control in every test run to monitor for reagent stability and correct assay performance. Please refer also to section 10.2\_Quality Control

### 8.0 SPECIMEN COLLECTION AND STORAGE

- Use serum in this procedure. It is most important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed.
- Obtain patient samples by non-traumatic venipuncture, using a vacuum tube or sterile syringe. If a syringe is used, transfer the blood immediately to a vacuum tube (plain red-top or serum separator).
- Allow samples to clot at room temperature (18...25°C) for at least 20-30 minutes, until the clot just begins to retract. Spin the sample in a centrifuge. Immediately following the centrifugation, transfer the cell-free serum to a tightly stoppered storage bottle.
- Do not use sera samples showing signs of haemolysis. If it is necessary to store a sample prior to analysis, it is recommended that, for a period of up to 72 hours, store the sample in a sealed container at 2...8°C. Freeze samples at -20°C if longer storage is required.
- Avoid repeat freeze-thawing.

## 9.0 ASSAY PROCEDURE

### 9.1 REAGENT PREPARATION

Bring all reagents to room temperature (18...25°C). Select sufficient microwells for the test. Remove protective covering and select sufficient wells to accommodate the patient samples, calibrators and assay controls. Each sample is recommended to be tested in duplicate.  
Dilute all serum samples and assay controls 1/100 in sample diluent by adding 10µl to 990µl sample diluent. Calibrators do not require dilution.

### 9.2 PIPETTING AND INCUBATION STEPS

A. Pipette 100µl of the calibrators, diluted control or diluted patient sample into the wells. To achieve blanking on the plate reader add a 'no serum' control of 100µl of sample diluent to the first well.

- B. Incubate the wells at room temperature (18...25°C) for 30 minutes.  
 C. Wash the wells three times as described in section 9.3\_ PROCEDURAL NOTES  
 D. Add 100µl of ready-to-use conjugate to each well.  
 E. Incubate the wells at room temperature (18...25°C) for 15 minutes.  
 F. Repeat washing as in section 6. above.  
 G. Add 100µl of ready-to-use TMB substrate to each well.  
 H. Incubate the wells at room temperature (18...25°C) for 15 minutes.  
 I. Add 50µl of stop solution to each well. Tap gently to ensure uniform color distribution and read within 15 minutes.  
 J. To read the plate, ensure the base is free from moisture and no air bubbles are in the wells. Read the absorbance of the well contents at 450nm and 405nm on a suitable plate reader. On readers equipped with a dual wavelength facility set the reference filter to 620nm.  
 L. Subtract the blank from the optical densities of the calibrator, controls and patient samples. If the assay was performed in duplicate, the mean of the wells should be taken.

### 9.3 PROCEDURAL NOTES

Do not allow the wells to dry between incubations.  
 Do not vary reagents and incubation temperatures above or below room temperature (18 - 25°C).

#### WASHING PROCEDURE

The washing procedure can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel.

### 10.0 CALCULATION OF RESULTS

#### 10.1 VALIDITY OF THE ASSAY

In order that the assay be valid, the following criteria must be fulfilled:

- Adaltis supplies with each EIAgen Combi 6 kit, positive and negative control samples which should be assayed with each run. The results of these quality control samples should fall within the limits indicated on the Certificate of Analysis.
- Results obtained for quality control sera must fall within acceptable ranges: please refer also to next section 10.2\_QUALITY CONTROL.

#### 10.2 QUALITY CONTROL

Good laboratory practice indicates that with each assay run, one or more quality control samples of known antibody level should be analysed as though they were clinical samples.

Should the results fall outwith this range the assay should be repeated using freshly prepared controls. Should the results continue to fall outside the specific range, and after equipment, adherence to the protocol and laboratory procedure have been verified, assistance from the supplier should be sought. Do not report patient results if the control results fall outwith the acceptable ranges.

#### 10.3 OD CONVERSION

The optical densities (ODs) higher than 2.0 are out of the measurement range of some microplate readers. It is therefore necessary, for ODs higher than 2.0, to perform a reading at 405nm (= wavelength of peak shoulder) in addition to 450nm (peak wavelength) in addition to 450nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm
- Read again the plate at 405 nm and 620 nm
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/Od 405 = 3.0), that is:  
 OD 450 nm = OD 405 nm x 3.0

Warning: the conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his own reader.

#### 10.4 DATA REDUCTION - SEMI-QUANTITATIVE CALCULATION

Calculate the absorbance value (OD) blank corrected for the kit calibrator and controls. Calculate the mean, blank corrected, absorbance value (OD) for duplicates of the patient samples. Using the following algorithm, calculate the concentration of each of the samples:

$$\frac{\text{Concentration of calibrator}}{\text{OD of calibrator}} \times \text{OD of sample or control} = \text{U/ml}$$

The concentration of each calibrator is 100 u/ml.

#### 11.0 EXPECTED VALUES

The assay cut-offs were determined by combining data from a panel of samples including normal samples and samples from people with an autoimmune condition. Details are shown in the table below:

Parameter	Total Samples	No. of Normal samples	No. of autoimmune samples	Age range of normals	No samples used in cut-off calculation
SS-A	100	22	78	18 - 60	78
SS-B	123	45	78	18 - 60	105
Sm	120	28	92	18 - 60	98
Sm/RNP	108	27	81	18 - 60	92
Jo-1	173	40	133	18 - 60	40
ScI-70	186	40	146	18 - 60	166

Arbitrary u/ml	Negative	Equivocal	Positive
SS-A/Ro	<10.0	10.0 - 15.0	>15.0
SS-B/La	<10.0	10.0 - 15.0	>15.0
Sm	<10.0	10.0 - 15.0	>15.0
Sm/RNP	<10.0	10.0 - 15.0	>15.0
Jo-1	<10.0	10.0 - 15.0	>15.0
ScI-70	<10.0	10.0 - 15.0	>15.0

It is recommended that equivocal results be retested using a subsequent sample. The Sm/RNP parameter measures antibodies to both the Sm and RNP antigens. The Sm antibody result should be taken into account when interpreting the result for RNP antibodies.

### 12.0 LIMITATIONS OF THE PROCEDURE

#### 12.1 KNOWN INTERFERENCES

Grossly haemolysed, lipaemic or microbiologically contaminated samples should not be used. Samples with abnormally elevated levels of haemoglobin, bilirubin and especially EDTA may interfere with assay performance and accuracy.

A 'hook effect' may only be seen with very high samples which are above the assay range. Since this is a semi-quantitative assay, any hook effect above the assay range will not influence the performance or accuracy. This is a sample specific effect.

#### 12.2 CAUTIONS IN INTERPRETATION OF THE RESULTS

A negative result should not be used as a sole criterion to rule out connective tissue disease or other autoimmune disease, but must be taken in relation to other clinical observations and diagnostic tests. While the precision of the EIAgen kit is sufficient to allow samples to be measured in single determinations, this is done at the clinical laboratory's discretion. It is advised that duplicate determinations should be used to enable identification of potential pipetting error or to allow for confirmation in the equivocal range.

It should be noted that ENA antibodies occur at low levels in other autoimmune and non-autoimmune conditions. Therefore all other clinical observations and diagnostic tests should be taken into account for clinical diagnosis.

### 13.0 PERFORMANCE CHARACTERISTICS

#### 13.1 PRECISION

Several samples were run in duplicate on three plates of the same batch. Typical results are shown below:

		Intra-assay variation			Inter-assay variation		
		A	B	C	A	B	C
SS-A	mean	100.2	95.7	58.5	93.5	142.0	0.7
	% CV	3.2	8.1	9.1	1.5	2.0	8.5
SS-B	mean	42.0	8.2	63.3	42.2	8.4	69.7
	% CV	3.5	4.3	2.8	0.5	13.8	9.8
Sm	mean	120.1	75.4	24.8	117.1	74.5	24.5
	% CV	1.4	0.1	0.6	2.5	1.8	4.8
Sm/RNP	mean	76.4	48.0	96.4	76.9	47.6	95.7
	% CV	3.1	2.8	3.7	0.6	1.5	1.2
Jo-1	mean	104.8	51.2	16.6	107.8	51.6	16.9
	% CV	3.3	4.0	2.6	5.5	3.1	6.4
ScI-70	mean	71.5	46.6	30.2	68.8	45.3	30.5
	% CV	0.6	7.7	1.8	3.6	2.2	2.1

#### 13.2 ANALYTICAL SPECIFICITY AND SENSITIVITY

For each parameter, a panel of 'normal' asymptomatic individuals was run (see table above for the sample size). The range of negative results was 97-100%.

No cross-reactivity with anti-dsDNA, anti-Thyroglobulin, anti-Thyroid Peroxidase, Rheumatoid Factor, anti-Gastric Parietal Cell, anti-Cardiolipin or ANCA was seen.

The sensitivity of the assay was established by calculation of the mean plus two standard deviations of 22 replicates of the "no serum" control which gave the following values:

SS-A/Ro:	0.8 u/ml	Sm/RNP:	0.7 u/ml
SS-B/La:	1.2 u/ml	Jo-1:	0.5 u/ml
Sm:	0.7 u/ml	ScI-70:	0.6 u/ml

#### 13.3 RELATIVE SPECIFICITY AND SENSITIVITY

Each assay was compared to another commercially available test and was found to be substantially equivalent. The results are shown below. Equivocal results were omitted from the calculations.

<b>SS-A</b>			
<b>Predicate</b>	<b>Positive</b>	<b>Equivocal</b>	<b>Negative</b>
Positive	18	0	0
Equivocal	3	0	0
Negative	1	0	78

**Relative sensitivity = 100.0 %**  
**Relative specificity = 98.7 %**  
**Overall agreement = 99.0 %**

<b>SS-B</b>			
<b>Predicate</b>	<b>Positive</b>	<b>Equivocal</b>	<b>Negative</b>
Positive	16	1	1
Equivocal	0	0	0
Negative	0	0	105

**Relative sensitivity = 94.1 %**  
**Relative specificity = 100 %**  
**Overall agreement = 99.2 %**

<b>Sm</b>			
<b>Predicate</b>	<b>Positive</b>	<b>Equivocal</b>	<b>Negative</b>
Positive	11	3	0
Equivocal	1	1	1
Negative	1	1	101

**Relative sensitivity = 100.0 %**  
**Relative specificity = 99.0 %**  
**Overall agreement = 99.1 %**

<b>Sm/RNP</b>			
<b>Predicate</b>	<b>Positive</b>	<b>Equivocal</b>	<b>Negative</b>
Positive	14	1	1
Equivocal	0	0	0
Negative	2	2	88

**Relative sensitivity = 93.3 %**  
**Relative specificity = 97.8 %**  
**Overall agreement = 97.1 %**

<b>Jo-1</b>			
<b>Predicate</b>	<b>Positive</b>	<b>Equivocal</b>	<b>Negative</b>
Positive	22	2	3
Equivocal	0	1	2
Negative	1	2	140

**Relative sensitivity = 88.0 %**  
**Relative specificity = 99.3 %**  
**Overall agreement = 97.6 %**

<b>Sci-70</b>			
<b>Predicate</b>	<b>Positive</b>	<b>Equivocal</b>	<b>Negative</b>
Positive	25	2	4
Equivocal	0	0	1
Negative	1	2	151

**Relative sensitivity = 86.2 %**  
**Relative specificity = 99.3 %**  
**Overall agreement = 97.2 %**

#### 14.0 AUTOMATION

Application protocols for the proper automation on the Adaltis microplate analyzers are available upon request at Adaltis directly.

#### 15.0 SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors .

<b>ERROR</b>	<b>POSSIBLE CAUSES / SUGGESTIONS</b>
OD very different ( $\pm$ 50%) from OD reported on QC	<ul style="list-style-type: none"> <li>- incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes)</li> <li>- incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance; note down the beginning of the incubation)</li> <li>- error in washing or in spectrophotometer reading (suggestion: check operating or settings of respective instruments)</li> <li>- contamination of Substrate (suggestion: use only disposable and clean plastic containers)</li> </ul>
Low reproducible results	<ul style="list-style-type: none"> <li>- incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes)</li> <li>- incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance)</li> <li>- error in washing or in reading to spectrophotometer (suggestion: check operating or settings of respective instruments)</li> <li>- contamination of Substrate (suggestion: use only disposable and clean plastic containers)</li> <li>- pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water)</li> </ul>
no colourimetric reaction after addition of substrate	<ul style="list-style-type: none"> <li>- no reagent pipetted</li> <li>- strong contamination of conjugate or Substrate</li> <li>- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)</li> </ul>
too low reaction (too low ODs)	<ul style="list-style-type: none"> <li>- incorrect conjugate (e.g. not from original kit)</li> <li>- incubation time too short, incubation temperature too low</li> </ul>
too high reaction (too high ODs)	<ul style="list-style-type: none"> <li>- incorrect conjugate (e.g. not from original kit)</li> <li>- accidental contamination/degradation of conjugate</li> <li>- incubation time too long, incubation temperature too high</li> <li>- water quality for wash buffer insufficient (low grade of deionization)</li> <li>- insufficient washing (conjugates not properly removed)</li> </ul>
unexplainable outliers	<ul style="list-style-type: none"> <li>- contamination of pipettes, tips or containers</li> <li>- insufficient washing (conjugates not properly removed)</li> </ul>
too high within-run CV%	<ul style="list-style-type: none"> <li>- reagents and/or strips not pre-warmed to Room Temp. prior to use</li> <li>- plate washer is not washing correctly (suggestion: clean washer head)</li> </ul>
too high between-run CV%	<ul style="list-style-type: none"> <li>- incubation conditions not constant (time, temperature)</li> <li>- controls and samples not dispensed at the same time (with the same intervals)</li> <li>- (check pipetting order)</li> <li>- person-related variation</li> </ul>

#### 16.0 BIBLIOGRAPHY

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