TECOmedical Group

TECO®

Urinary Collagen IV ELISA

Kit instruction
English

Catalogue No. TE 1054
For Research Use Only

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Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

TE 1054

Caution: caustic

Intended use

Tests

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TECO® Urinary Collagen IV ELISA Kit

CONT **Reagents and Materials Supplied:**

SYMBOL	DESCRIPTION	FORMAT
1	**Collagen IV Antibody coated Microassay Plate** 12 strips of 8 wells (96 break apart wells in total), in a frame. Ready to use.	**1 Plate**
A	**Collagen IV Calibrator – Standard A** Ready to use. 0.8 µg/L	**1 x 1.0 ml**
B	**Collagen IV Calibrator – Standard B** Ready to use. 3.2 µg/L	**1 x 1.0 ml**
	Collagen IV Calibrator – Standard C Ready to use. 12.5 µg/L	**1 x 1.0 ml**
D	**Collagen IV Calibrator – Standard D** Ready to use. 50 µg/L	**1 x 1.0 ml**
2	**Wash Buffer: 10x concentrate**	**2 x 50 ml**
3	**Assay Buffer** Ready to use.	**1 x 10 ml**
6	**Enzyme Conjugate: Anti-collagen IV mouse Fab' conjugated to horseradish peroxidase** Ready to use.	**1 x 20 ml**
7	**TMB Substrate** Ready to use.	**1 x 15 ml**
8	**Stop Solution: 1M Sulphuric Acid** Ready to use.	**1 x 15 ml**
	Plate Seal	**1 sheet**
	Kit instructions	**1 x**

Storage

Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

INTENDED USE

The TECO®Urinary Collagen IV ELISA provides a method for the quantitative determination of collagen IV in human urine. Please contact TECOmedical for further information regarding the assay of collagen IV in other tissue fluids. The TECO®Urine Collagen IV ELISA is for research use only. Not for use in diagnostic procedures.

BACKGROUND

Type IV collagen (IV•C), is a major component of the basement membrane (BM), and it is considered to constitute its basic framework. Urinary collagen IV levels are elevated in a variety of renal pathologies[1], particularly diabetic nephropathy[2-8]. Urinary collagen IV is significantly higher in patients with non-insulin dependent diabetic mellitus (NIDDM) than in normal subjects and urinary collagen IV levels correlate with the deposition of collagen IV in the kidney[2]. In diabetic subjects, urinary collagen IV is significantly increased in patients with microalbuminuria or overt proteinuria as compared to those with normoalbuminuria[3] (Figure 1). Moreover, in diabetics with normoalbuminuria, those with elevated urinary collagen IV were at an increased risk for progression to microalbuminuria[4]. Intensive therapy of diabetic nephropathy can slow the temporal increase in urinary collagen IV in diabetics indicating the potential of urinary collagen IV for studying the renal effects of new therapies[5]. These results suggest that the measurement of UIV•C might provide a useful biomarker for studying diabetic nephropathy.
In the field of renal transplantation, renal collagen IV levels are increased[9] and increased urinary collagen IV levels are found in acute renal rejection[10], indicating the potential value of urinary collagen IV in studying these conditions.

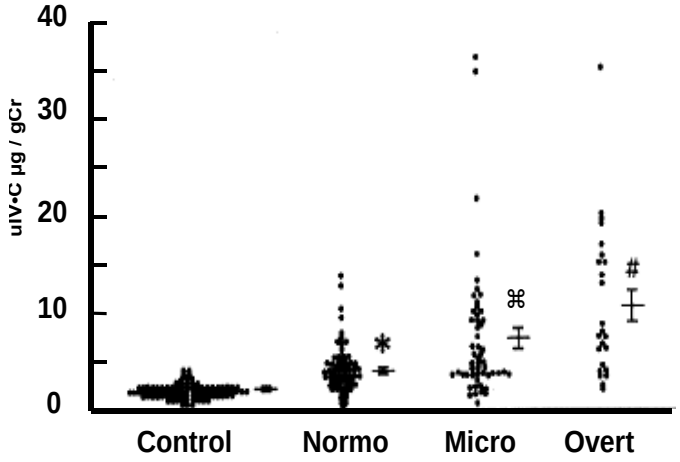

Figure. 1. Urinary collagen IV in diabetic subjects with various stages of diabetic nephropathy[3].

Control; n=89, Normo (normoalbuminuria); n=62, Micro (microalbuminuria); n=53, Overt (overt proteinuria); n=24, mean±SE. ✱ p<0.01 vs Control, ⌘ ? p<0.05 vs Normo, # p<0.05 vs Micro.

REFERENCES

1) **Makino, H. *et al*. (1995).** Urinary detection of type IV collagen and its increase in glomerulonephritis. Research Communications in Molecular Pathology and Pharmacology. **88(2),** 215-223.
2) **Okonogi, H. *et al.* (2001).** Urinary type IV collagen excretion reflects renal morphological alterations and type IV collagen expression in patients with type II diabetes mellitus. Clinical Nephrology. **55,** 357-364.
3) **Isono, M. *et al*. (1996).** Analysis of urinary type IV collagen in NIDDM subjects as a marker for diabetic nephropathy. J. Japan. Diab. Soc. **39**, 599-604.
4) **Ieki, Y. and Takazakura, E. (1999)** Analysis of urinary excretion of type IV collagen as a predictor of progression of early diabetic nephropathy. – Two year follow up study. J. Japan. Diab. Soc. **42(10)**, 859-862.
5) **Nishimura, M. *et al* (2001).** Angiotensin converting enzyme inhibitors and Probucol suppress the time-dependent increase in urinary type IV collagen excretion of type II diabetes mellitus patients with early diabetic nephropathy. Clinical Nephrology. **55(2)** 96-103.
6) **Ohgaku, S. *et al*.** (**1996).** Direct determination of concentrations of urinary type IV collagen in normal and diabetic subjects with a sensitive enzyme immunoassay (EIA). J. Japan. Diab. Soc. **39**, 523-526, 1996.
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8) **Yagame, M. *et al*. (1997).** Significance of urinary type IV collagen in patients with diabetic nephropathy using a highly sensitive one-step sandwich enzyme immunoassay. J. Clin. Lab. Anal. **11**, 110-116.
9) **Kawase, T. *et al*. (2001).** Collagen IV is upregulated in chronic transplant nephropathy. Transplantation Proceedings. **33,** 1207-1208.
10) **Haddad, C. *et al*. (1996).** Elevated urine collagen IV levels (UC.IV) correlate with the severity of acute renal transplant (TX) rejection (AR). Poster 196 presented at the 1996 ATSP meeting.
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ASSAY PRINCIPLE

The TECO®Urinary Collagen IV ELISA is designed for the assay of urinary collagen IV. It is a solid phase one-step sandwich ELISA. Collagen IV in the sample is bound simultaneously by a solid phase monoclonal antibody and a monoclonal antibody-enzyme conjugate directed at different antigenic sites. This results in the collagen IV molecule being sandwiched between the solid phase and enzyme labelled antibodies. After removing unbound enzyme labelled antibody and sample, the plate is then incubated with enzyme substrate, resulting in the development of a colour. The colour developed is directly proportional to the amount of collagen IV in the sample.

Materials Required and not Supplied

- Pipette 50 µL
- Multichannel pipettes for 100-150 µL
- Graduated cylinders for reconstituting or diluting reagents
- Manual Aspiration System or Automatic washer for ELISA plates
- Aqua dest.
- Vortex mixer
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm (Reference: 590-650 nm)
- ELISA plate shaker (500 rpm) (orbital shaker)
- Software package for data generation and analysis

Warnings and Precautions

This kit is intended for research use by professional persons only.
Follow the instructions carefully.

Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to ”Materials Safety Data Sheet” for more detailed safety information.

Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

Material of human origin used in the preparation of this kit has been tested and found non reactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1 For research use only.
2 Treat all specimen samples as potentially biohazardous material.
Follow General Precautions when handling contents of this kit and any patient samples.
3 Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
4 Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5 Store assay reagents as indicated.
6 Do not use coated strips if pouch is punctured.
7 Test each sample in duplicate.
8 Use of multichannel pipettes is recommended to ensure the timely delivery of liquids.
9 a. 1 M sulphuric acid is caustic and can be harmful for skin, eyes and mucosae.
b. Handle TMB with care. Do not ingest. Avoid contact with skin, eyes, or clothing. Should there be any contact, wash with water. If ingested, call a physician.
10 A mercury-free preservative is used. Incidental contact with or ingestion of buffer solutions may cause irritation of skin, eyes or mouth. Should there be any contact, wash with water. If ingested, call a physician.

PROCEDURAL

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges specified may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Reagent delivery should be aimed at midpoint of the side of the wells, taking care not to scratch the side with the pipette tip.
- Do not allow the wells to dry at any stage during the assay procedure.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not use reagents that are cloudy or that have precipitated out of solution.
- Ensure Wash Concentrate is mixed thoroughly and no crystals remain before reconstitution.
- High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background colour in the assay.
- Allow all reagents to come to 20-27°C and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay, an identification and distribution plan should be established.

Stability and Storage

1. All kit reagents should be stored at 2-8°C and are stable as supplied until the expiry date shown.
2. Prepared Wash Solution (PBT) is stable for up to one month at 2-8°C.
3. Plate assay wells should be stored in sealed bags with desiccants at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.
4. Do not store urine samples without the addition of Urine Stabilizing Buffer (USB). USB must be added within 12 hours of sample collection.
   As soon as possible after sample collection, add 100µL of Urine Stabilizing Buffer (TE1050 or TE1055) to 400µL urine (4/5 dilution of sample), even if the samples are not to be stored.

PREPARATION OF REAGENTS

WASH SOLUTION (PBT)

Perform a 1/10 dilution of Wash Concentrate adding, for example, 10 mL Wash Concentrate to 90 mL deionised water as required. Prepare only the volume of Wash Solution required for the assay. Each row of assay wells requires 15 mL of Wash Solution.

Ensure salt crystals are dissolved prior to dilution.

Gentle warming of Wash Concentrate at 37°C for 30 minutes will aid dissolution of salt crystals.

SAMPLE COLLECTION AND STORAGE

Collagen IV precipitates out of urine upon standing leading to falsely low results. This can be prevented by the addition of a stabilizing buffer to the urine after which the urine can be stored[11] (TE1050 or TE1055).

Transfer urine to the collection tube using a Pasteur pipette. Fill the tube as indicated in the instructions, then mix thoroughly. As collagen IV is absorbed by urinary precipitates that form during storage, collect fresh urine and use the TECO Urine Stabilizing Buffer (TE1050 or TE1055) for sample stabilization (see Stability and Storage). Urine samples must be transferred to the collection tubes on the day of collection. To facilitate compensation for dieresis, it is recommended that a simultaneous sample be taken for urinary creatinine.

After addition to the urine tubes, samples can be stored at 2-8°C for one week or nine months at –20°C. If samples have been frozen, it is essential to mix thoroughly to dissolve any precipitates. Repeated freeze thawing of samples should be avoided

ASSAY PROCEDURE

NOTE: All reagents should be allowed to reach room temperature prior to commencement of assay.

1. IMMUNOREACTION

1.1 Prepare Wash Solution as described in "Preparation of Reagents".
1.2 Place required number of Microassay wells in the assay plate (10 for the Calibrators plus two for each sample).
1.3 Add **150 µL** Conjugate to each well using a multichannel pipette.
1.4 Add Calibrators **(0 (Assay buffer), 0.8, 3.2, 12.5 and 50 µg/L)** and samples **(50 µL/well)**, in duplicate, to the Microassay plate.

1.5 Cover the Microassay plate with the plate seal and incubate at 20-27°C for **24 hours**.

1.6 Remove plate seal cover and wash each strip five times **(350 µL/well)** with 1x Wash Buffer. When complete, firmly tap the plate against a paper towel to ensure complete removal of wash fluid from wells.

2. COLOUR DEVELOPMENT

2.1 Add **100µL** Substrate/well using a multichannel pipette and incubate at 20-27^{o}C for exactly **one hour**.

3. STOP

3.1 Add **100 µL** Stop Solution/well using a multichannel pipette. Ensure complete mixing of Substrate and Stop Solution using a plate shaker.
3.2 Read **immediately** at 450 nm using 630 nm as reference (if available).

CALCULATION OF RESULTS

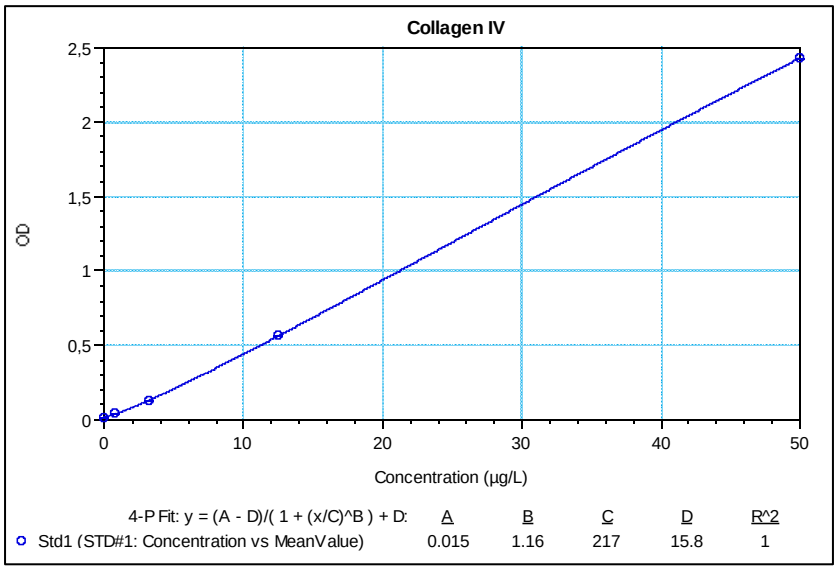
1. Calculate the mean absorbance for each calibrator and sample.
2. Plot a Calibration curve of $A_{450/630nm}$ versus collagen IV (µg/L) on a log-log scale.
3. Read the collagen IV (µg/L) indicated by the mean absorbances of the samples from the calibration curve.
4. If the samples have been diluted, multiply the calculated [collagen IV] by the appropriate dilution factor in order to obtain the actual [collagen IV].

Please note:
Multiply the calculated collagen value by the appropriate dilution factor in order to obtain the actual collagen. Results for stabilized urine samples should be multiplied by an additional factor of 1.25 to compensate for the dilution of sample with Urine Stabilizing Buffer.

Example of Calibration Curve

(Example only, not for use in calculation of actual results)

Standard	Concentration (µg/L)	OD (450 nm)
Assay Buffer	0	0.012
A	0.8	0.043
B	3.2	0.128
C	12.5	0.563
D	50.0	2.430



PERFORMANCE CHARACTERISTICS

NORMAL RANGE

Based on healthy Japanese volunteers, 95% confidence limits for urinary collagen IV are:

		Collagen IV µg/g creatinine	N
Early morning urine	30-39 years	<4.0	122
	>40 years	<4.9	64
Spot urine	<21 years	<7.3	390

LIMIT OF DETECTION

The detection limit of the TECO®Urine Collagen IV ELISA is 0.8 µg/L.

MEASURING RANGE

The calibration curve range covers the range 0.8-50 µg/L. This range may be extended by increasing sample dilution.

SPECIFICITY

The TECO®Urine Collagen IV ELISA is highly specific for the detection of collagen IV. Cross reactivity is less than 2% with Collagen II and less than 0.5% with other forms of collagen.

SENSITIVITY

When reading from the standard curve the A_{450nm} value of the 0.8 µg/L standard should be 0.01 – 0.06, and the 50 µg/L standard should be >0.8.

INTERFERENCE

No significant interference has been observed in this assay with creatinine, haemolytic or icteric samples.

- Creatinine: Less than 10% interference up to 3 g/L in sample.
- Haemolysis: Less than 10% interference up to 4.8 g/L haemoglobin.
- Icteris: Less than 10% interference up to 0.2 g/L bilirubin.

DILUTION - RECOVERY

Dilution of samples containing high levels of collagen IV gave the following results:

Sample	Dilution								
	1 / 2			1 / 4			1 / 8		
	Expected µg/L	Obtained µg/L	Recovery %	Expected µg/L	Obtained µg/L	Recovery %	Expected µg/L	Obtained µg/L	Recovery %
A	11.6	11.0	95	5.8	5.2	90	2.9	2.6	90
B	13.4	12.7	95	6.7	6.4	96	3.3	3.1	94
C	5.1	5.4	106	2.6	2.5	96	1.3	1.3	100

REPRODUCIBILITY

Intra-assay variation of the TECO®Urinary Collagen IV ELISA.

Sample	Collagen IV µg/L	SD	% CV	N
Low	2.5	0.07	2.8	8
Medium	6.2	0.13	2.1	8
High	10.8	0.29	2.7	8

Inter-assay variation of the TECO®Urinary Collagen IV EIA

Sample	Collagen IV µg/L	SD	%CV	N
Low	2.4	0.05	2.1	4
Medium	6.0	0.15	2.0	4
High	20.8	1.52	7.3	4

Inter-batch Variation of the TECO®Urinary Collagen IV ELISA calculated for four batches of kits.

Sample	Collagen IV µg/L	SD	%CV	N
Low	3.2	0.18	5.6	4
Medium	5.8	0.11	1.9	4
High	17.0	0.52	3.1	4

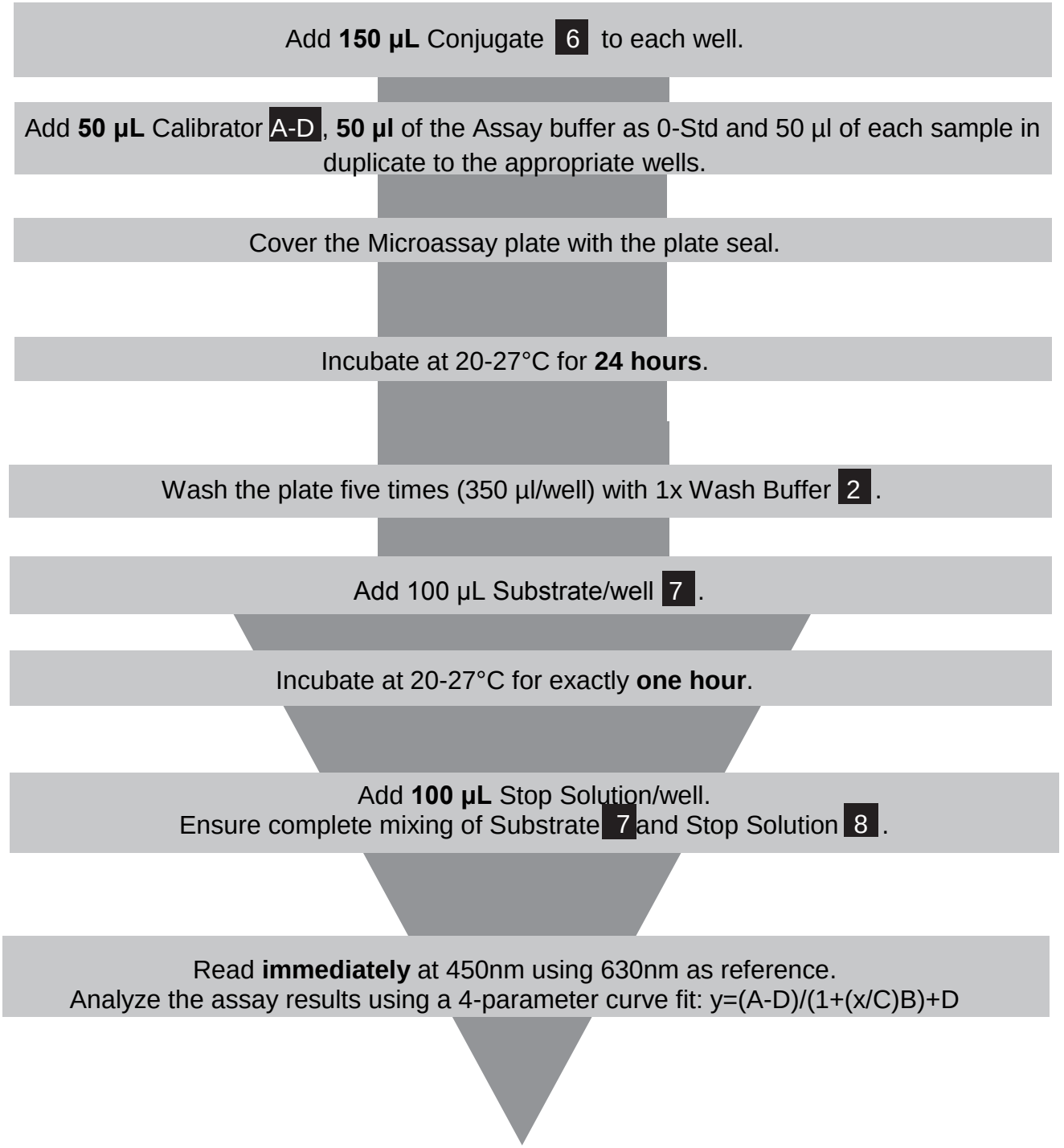
NOTE:

NOTE:

TECO® Urinary Collagen IV ELISA Kit

Assay Procedure – Quick Guide

- Bring samples and reagents to 20-27°C. Mix the samples well.
- Dilute Wash Buffer 2 1:10 with deionized or destilled water.
- Prepare the required number of Assay strips 1 .



Please read the Kit Instructions before using the Quick Guide.