Instructions for Use

For the quantitative measurement of HMW Kininogen in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.
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INTRODUCTION

1. BACKGROUND

Abcam’s HMW Kininogen Human in vitro SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of HMW Kininogen protein in serum, plasma and cell culture supernatants.

The SimpleStep ELISA™ employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

High molecular weight kininogen (HMW kininogen) is a 72kDa highly glycosylated protein with an important role in the assembly of the plasma kallikrein-kinin system and in the blood coagulation process. It is encoded by the KNG1 gene, which generates both HMWK and low molecular weight kininogen (LMW kininogen) via alternative splicing. Both HMW kininogen and LMW kininogen share a heavy chain consisting of protein domains 1, 2 and 3 and differ in their light chain. HMW kininogen contains a 56kDa light chain consisting of domain 5 and 6H, whereas LMW kininogen contains a 4kDa light chain consisting of domain 5L. Heavy and light chains of HMW kininogen and LMW kininogen are linked via domain 4 which contains the bradykinin nonapeptide.
INTRODUCTION

HMW kininogen is mainly secreted by the liver and helps position optimally prekallikrein and factor XI next to factor XII. Positioning of prekallikrein in contact with factor XII results in activation and cleavage of factor XII into factor XIIa. This leads to a positive feedback mechanisms of contact activation and cleavage of HMW kininogen with the release of bradykinin. Active bradykinin affects smooth muscle contraction, induces hypotension, natriuresis, diuresis, decreases blood glucose level, mediates inflammation by releasing prostaglandins, increases vascular permeability and stimulates nociceptors. Furthermore, HMW kininogen also inhibits the thrombin- and plasmin-induced aggregation of thrombocytes.

HMW kininogen deficiency is an autosomal recessive coagulation defect known as Fitzgerald trait, Flaujeac trait, Fujiwara trait, Reid trait or Williams’s trait. Patients with low levels of HMW kininogen exhibit abnormal surface-mediated activation of fibrinolysis noted by prolonged partial thromboplastin time (PTT), normal prothrombin time (PT) and normal thrombin time typically found during a preoperative screening workup in asymptomatic individuals. A SNPs in KNG1 gene (rs710446) has been significantly associated with shortened activated partial thromboplastin time (aPTT) increasing the risk of venous thrombosis.
2. **ASSAY SUMMARY**

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.

Add standard or sample to appropriate wells.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X HMW Kininogen Capture Antibody</td>
<td>2 x 300 µL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>HMW Kininogen Detector Antibody (Lyophilized)</td>
<td>2 Vials</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>HMW Kininogen Human Lyophilized Purified Protein</td>
<td>2 Vials</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Antibody Diluent 5BI</td>
<td>2 x 3 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>10X Wash Buffer PT</td>
<td>20 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>5X Cell Extraction Buffer PTR*</td>
<td>10 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>50X Cell Extraction Enhancer Solution*</td>
<td>1 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Sample Diluent NS</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Pre-Coated 96 Well Microplate (12 x 8 well strips)</td>
<td>96 Wells</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Plate Seal</td>
<td>1</td>
<td>+2-8°C</td>
</tr>
</tbody>
</table>

* 5X Cell Extraction Buffer PTR and 50X Cell Extraction enhancer solution only required for cell and tissue samples.
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm
- Method for determining protein concentration (BCA assay recommended)
- Deionized water
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4)
- Multi- and single-channel pipettes
- Tubes for standard dilution
- Plate shaker for all incubation steps
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors)

7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. **TECHNICAL HINTS**

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers
- Avoid foaming or bubbles when mixing or reconstituting components
• Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
• Ensure plates are properly sealed or covered during incubation steps
• Complete removal of all solutions and buffers during wash steps is necessary to minimize background
• As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11)
• All samples should be mixed thoroughly and gently
• Avoid multiple freeze/thaw of samples
• Incubate ELISA plates on a plate shaker during all incubation steps
• When generating positive control samples, it is advisable to change pipette tips after each step
• The provided 5X Cell Extraction Buffer contains phosphatase inhibitors and protease inhibitor aprotinin. Additional protease inhibitors can be added if required
• The provided Antibody Diluents and Sample Diluents contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required
• The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation
• To avoid high background always add samples or standards to the well before the addition of the antibody cocktail
• This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions
9. **REAGENT PREPARATION**

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 **1X Wash Buffer PT**

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 **10X HMW Kininogen Detector Antibody**

To reconstitute the lyophilized HMW Kininogen Detector Antibody, centrifuge the vial at 10,000 x g for 2 minutes and then add 160 µL of nanopure water per vial; incubate at room temperature for 5 minutes and mix well ensuring the material is fully resuspended. Add 160 µL Sample Diluent NS and mix at room temperature for 5 minutes on a tube rotator.

*Note: For long term storage replace Sample Diluent NS for glycerol and store at -20°C.*

9.3 **Antibody Cocktail**

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.
9.4 1X Cell Extraction Buffer PTR (For cell and tissue extracts only)

If required, prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 μL 50X Cell Extraction Enhancer Solution. Mix thoroughly and gently. If required protease inhibitors can be added.

*Alternative* – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in Section 19.
10. **STANDARD PREPARATION**

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following table describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the HMW Kininogen standard sample by adding 1 mL Sample Diluent NS by pipette. Mix thoroughly and gently. Hold at room temperature for 3 minutes and mix gently. This is the 20,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tube, Standards 1–8.

10.3 Add 370 µL Sample Diluent NS into tube number 1 and 150 µL Sample Diluent NS into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

```
20,000 pg/mL
1,500 pg/mL
750 pg/mL
375 pg/mL
187.5 pg/mL
93.75 pg/mL
46.88 pg/mL
23.44 pg/mL
```

![Diagram of standard preparation]

![Diagram of standard preparation]
ASSAY PREPARATION

11. SAMPLE PREPARATION

<table>
<thead>
<tr>
<th>TYPICAL SAMPLE DYNAMIC RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
</tr>
<tr>
<td>Human Serum</td>
</tr>
<tr>
<td>Human Plasma - Citrate</td>
</tr>
<tr>
<td>Human Plasma - EDTA</td>
</tr>
<tr>
<td>Human Plasma - Heparin</td>
</tr>
<tr>
<td>Cell Culture Media</td>
</tr>
</tbody>
</table>

11.1 Plasma
Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum
Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants
Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
12. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

*Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:*
<table>
<thead>
<tr>
<th>Mode</th>
<th>Kinetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength:</td>
<td>600 nm</td>
</tr>
<tr>
<td>Time:</td>
<td>up to 15 min</td>
</tr>
<tr>
<td>Interval:</td>
<td>20 sec - 1 min</td>
</tr>
<tr>
<td>Shaking:</td>
<td>Shake between readings</td>
</tr>
</tbody>
</table>

*Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.*

13.9 Analyze the data as described below.
14. **CALCULATIONS**

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
15. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![TYPICAL STANDARD CURVE](image)

**Standard Curve Measurements**

<table>
<thead>
<tr>
<th>Conc. (pg/mL)</th>
<th>O.D. 450 nm</th>
<th>Mean O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>0.065</td>
<td>0.064</td>
</tr>
<tr>
<td>23.44</td>
<td>0.041</td>
<td>0.114</td>
</tr>
<tr>
<td>46.88</td>
<td>0.161</td>
<td>0.137</td>
</tr>
<tr>
<td>93.75</td>
<td>0.230</td>
<td>0.193</td>
</tr>
<tr>
<td>187.5</td>
<td>0.303</td>
<td>0.287</td>
</tr>
<tr>
<td>375</td>
<td>0.485</td>
<td>0.506</td>
</tr>
<tr>
<td>750</td>
<td>0.547</td>
<td>0.897</td>
</tr>
<tr>
<td>1,500</td>
<td>1.577</td>
<td>1.602</td>
</tr>
</tbody>
</table>

**Figure 1.** Example of HMW Kininogen standard curve. The HMW Kininogen standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.
16. **TYPICAL SAMPLE VALUES**

**SENSITIVITY –**

The calculated minimal detectable dose (MDD) is 8.7 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentrations.

**RECOVERY –**

Three concentrations of HMW Kininogen were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Serum (1:70,000)</td>
<td>97</td>
<td>81 – 109</td>
</tr>
<tr>
<td>Human Plasma - EDTA (1:300,000)</td>
<td>119</td>
<td>111 – 130</td>
</tr>
<tr>
<td>Human Plasma – Citrate 1:300,000</td>
<td>116</td>
<td>95 – 135</td>
</tr>
<tr>
<td>Human Plasma - Heparin (1:300,000)</td>
<td>109</td>
<td>101 – 115</td>
</tr>
<tr>
<td>RPMI media (1:10)</td>
<td>99</td>
<td>91 – 109</td>
</tr>
</tbody>
</table>

**LINEARITY OF DILUTION –**

Native HMW Kininogen was measured in serum and plasma samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS. Recombinant HMW Kininogen was spiked into RPMI media and diluted in a 2-fold dilution series in Sample diluent NS.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.
### DATA ANALYSIS

#### Dilution Factor

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>Interpolated value</th>
<th>0.0006% Human Serum</th>
<th>0.003% Human Plasma (Citrate)</th>
<th>0.003% Human Plasma (EDTA)</th>
<th>0.003% Human Plasma (Heparin)</th>
<th>10% RPMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pg/mL</td>
<td>448.81</td>
<td>2,060.8</td>
<td>2,340.2</td>
<td>1,721.30</td>
<td>686.4</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>pg/mL</td>
<td>223.82</td>
<td>990.23</td>
<td>1,057.4</td>
<td>778.95</td>
<td>351.7</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>100</td>
<td>96</td>
<td>90</td>
<td>91</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>pg/mL</td>
<td>112.69</td>
<td>501.01</td>
<td>535.79</td>
<td>383.17</td>
<td>170.7</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>100</td>
<td>97</td>
<td>92</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>pg/mL</td>
<td>59.55</td>
<td>251.28</td>
<td>266.69</td>
<td>192.57</td>
<td>84.9</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>106</td>
<td>98</td>
<td>91%</td>
<td>90</td>
<td>99</td>
</tr>
<tr>
<td>16</td>
<td>pg/mL</td>
<td>32.70</td>
<td>133.38</td>
<td>149.71</td>
<td>103.61</td>
<td>46.5</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>117</td>
<td>104</td>
<td>102</td>
<td>96</td>
<td>108</td>
</tr>
</tbody>
</table>

### PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of Serum within the working range of the assay.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.5</td>
<td>7.4</td>
</tr>
</tbody>
</table>
SAMPLE DATA –

Figure 2. Ten individual healthy donors were evaluated for the presence of HMW Kininogen in serum using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected by sample dilution (1:1,000,000). The mean level of HMW Kininogen is 58.2 µg/mL with a range of 42.8 – 69.5 µg/mL and a standard deviation of 8.3 µg/mL.

17. ASSAY SPECIFICITY
This kit recognizes both native and recombinant Human HMW Kininogen protein in serum, plasma and culture media samples only. Cell culture and tissue homogenate samples have not been tested with this kit.

CROSS REACTIVITY -
The proteins listed below were prepared at 25 ng/mL in Sample Diluent NS assayed for cross-reactivity. No significant cross-reactivity was observed with a mean OD deviation from background of 0.025 ODs.

Recombinant Human:
LMW Kininogen
**INTERFERENCE -**
Recombinant HMW Kininogen was assayed at 250 pg/mL in the presence and absence of 25 ng/mL of LMW Kininogen to determine interference. Recovery of HMW Kininogen was observed at 95.3%.

**18. SPECIES REACTIVITY**
This kit recognizes Human HMW Kininogen protein.

Other species reactivity was determined by measuring 1:1,000,000 (dilution) serum samples of various species, interpolating the protein concentrations from the Human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in Human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:
- Mouse
- Rat
- Hamster
- Guinea Pig
- Rabbit
- Dog
- Goat
- Pig
- Cow

Please contact our Technical Support team for more information.
## 19. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficulty pipetting lysate; viscous lysate.</td>
<td>Genomic DNA solubilized</td>
<td>Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.</td>
</tr>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate Pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standard dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td></td>
<td>Incubation times with TMB too brief</td>
<td>Ensure sufficient incubation time until blue color develops prior addition of Stop solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.</td>
</tr>
<tr>
<td>Precipitate in Diluent</td>
<td>Precipitation and/or coagulation of components within the Diluent.</td>
<td>Precipitate can be removed by gently warming the Diluent to 37°C.</td>
</tr>
</tbody>
</table>
20. **NOTES**
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

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Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

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