Circulating Immune Complexes EIA
CIC-C1q

This kit is intended for the quantitative determination of circulating immune complexes (CIC) in serum or plasma of patients with various autoimmune and other CIC–related diseases

For In Vitro Diagnostic Use

Catalog Number: 01-EK -CIC
Size: 96 tests
Storage: 2 – 8°C
Manufactured by: Bühlmann Laboratories
Switzerland
I. INTENDED USE

This kit is intended for the quantitative determination of circulating immune complexes (CIC) in serum or plasma of patients with various autoimmune and other CIC-related diseases.

II. SUMMARY AND EXPLANATION

Circulating Immune Complexes (CIC) are formed by the interaction of antibodies with immunogenic antigens. Immune complexes with only a slight excess of antibody or antigen are soluble and activate complement. CIC formation may be viewed as a host defense against foreign antigens. Under normal circumstances CIC are cleared by phagocytosis. If CIC escape phagocytic clearance they may be deposited in endothelial or vascular structures provoking an inflammatory damage by tissue response. CIC have been associated with an increasing variety of diseases (1,2), covering autoimmune, rheumatic, renal and many other diseases:

Autoimmune and Rheumatic Diseases
Systematic Lupus Erythematosus (SLE), rheumatoid arthritis (RA) and vasculitides.

Renal
Iodopathic glomerulonephritis, post-streptococcal glomerulonephritis and IgA nephropathy.

The C1q solid phase assay is particularly suitable for monitoring the disease state of Systemic Lupus Erythematosus (SLE) patients: CIC values obtained with this assay show a good correlation with high titers of dsDNA antibodies and with depressed total complement activity (3). The measurement of CIC maybe useful in therapeutic monitoring (4,5).

III. PRINCIPALS OF THE ASSAY

Circulating Immune Complexes from patient sera, plasma or standards and controls are incubated with human C1q adsorbed onto microtiter wells. After a washing step, an alkaline phosphatase labelled conjugate is added, which binds to the Fc region of human IgG. After another washing step, the enzyme substrate is pipetted followed by a stopping step (6,7,8). The absorption is measured at 405 nm.

IV. REAGENTS SUPPLIED AND STORAGE

1. Microtiter plate – Divisible (12 strips, 8 wells each) precoated with human C1q. Ready to use.
   
   Stable at 2-8° C until expiration date (exp. date). Protect unused strips from moisture by storing with desiccant and carefully resealing plastic bag.

2. Plate Sealer – 3 pieces.

3. Standards A to E – 5 x 1.0 ml aggregated-IgG in a buffer matrix with preservatives. The Standards A, B, C, D and E contain 50, 20, 10, 4 and 1 µg Equivalents of aggregated-IgG per ml (µg Eq/ml) respectively. Ready to use.
   
   Stable at 2-8° C until exp. date. After opening stable for up to 1 month at 2-8° C or until exp. date at –20 °C.

4. Controls – 2 x 1.0 ml (low and high) controls of aggregated-IgG in a buffer matrix with preservatives. The controls contain lot-specific amount of Equivalents of aggregated-IgG per ml (µg Eq/ml). Refer to the additional Control Sheet for exact concentrations. Ready to use.
   
   Stable at 2-8° C until exp. date. After opening stable for up to 1 month at 2-8° C or until exp. date at –20 °C.

5. Incubation Buffer – 1 x 100 ml. Ready to use (turbid suspension, colorless).
   
   Stable at 2-8° C until exp. date after opening.

6. Enzyme Label – 1 x 11 ml protein A conjugated to alkaline phosphatase in a protein-based buffer matrix with preservatives. Ready to use (yellow solution).
   
   Stable at 2-8° C until exp. date after opening.

7. Substrate Solution – 1 x 11 ml para-nitrophenyl-phosphate (pNPP). Ready to use (colorless).
   
   Stable at 2-8° C until exp. date after opening.

8. Stop Solution – 1 x 11 ml 1 N sodium hydroxide. Ready to use.
   
   Stable at 2-8° C until exp. date after opening.

This protocol is for reference purposes only. DO NOT use this copy to run your assay; use the protocol included with the kit ONLY.
9. **Wash Buffer** – 1 x 100 ml concentrate with preservatives. Dilute with 900 ml of deionized water to prepare 1000 ml of Wash Buffer. Stable for up to 6 months at 2-8°C until expiration date after opening.

### V. PRECAUTIONS

**FOR IN VITRO DIAGNOSTIC USE ONLY.** Not for internal and external use in humans or animals.

**General Precautions**

Concerning the proper precautions for the handling and disposal of kit reagents and patient specimens, respectively, we highly recommend to first consult the special local regulations of your country. Some of these basic safety rules are listed below:

- **Cover working area with disposable absorbent paper.**
- **No food, beverages, cosmetics or tobacco products should be admitted in areas where patient specimens or kit reagents are handled.**
- **Wear disposable gloves while handling patient specimens and kit reagents, wash hands afterwards.**
- **Do not pipet any reagent by mouth.**

**Reagents Containing Human Source Material**

The Microtiter Strips and Standards of this kit contain components of human origin. **All patient specimens and kit components should be handled as if capable of transmitting infections.** Those products should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health Manual, *Biosafety in Microbiological and Biomedical Laboratories*, 3rd ed., 1993. HHS Publication No. (CDC) 93-8395.

**Reagents Containing Sodium azide and Thimerosal**

This kit **does not** include reagents containing Sodium Azide or Thimerosal as a preservative.

**Substrate and Stop Solution**

The Substrate Solution contains para-Nitrophenyl-Phosphate (pNPP). The Stop Solution contains Sodium Hydroxide. Each of those reagents is irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. After contact with eyes or skin, wash immediately with plenty of water.

### VI. MATERIALS REQUIRED BUT NOT PROVIDED

- 20 µl, 100 µl and 1000 µl precision pipettes with disposable tips.
- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions.
- 1000 ml cylinder for the dilution of the Wash Buffer Concentrate.
- Microtiter plate washer or squeeze bottle for Wash Buffer.
- Microtiter plate rotator (ALPCO cat # 006-AL-RP1).
- Microtiter plate reader for measurement of absorbance at 405 nm.

### VII. SPECIMEN COLLECTION AND STORAGE

- Collect blood by venipuncture into tubes, avoid hemolysis, mix by inverting sample tube several times and leave to clot for 45 minutes at room temperature (18-28°C) protected from light. Centrifuge at 1800 x g for 15 minutes at room temperature (18-28°C) and collect the serum. Lipemic, hemolytic and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking patients to fast for at least 12 hours prior to the sample being taken. The procedure calls for about 100 µl blood per duplicate determination. **Do not heat-inactivate samples.**
- Samples may be stored at 2-8 °C for up to 7 days. If samples are to be stored for a longer period of time, they should be kept at -20 °C or lower.

### VIII. PROCEDURAL NOTES

**Allow the reagents to come to 18-28°C prior to use**

1. Dilute all patient samples 1:50 with Incubation Buffer (e.g. 20 µl of serum or plasma + 980 µl of Incubation Buffer) and mix well. Allow diluted samples to stand for 15 minutes at 18-28°C prior to pipetting in step 4c.
2. Prepare a plate with sufficient strips to test the required number of standards, controls and samples. Remove excess strips from the holder and re-seal them in the foil pouch together with the desiccant packs **without delay.** Store refrigerated.
3. Wash the coated wells twice using at least 350 µl of Wash Buffer perwell. Empty the wells and strike the plate firmly onto blotting paper.

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4a. Pipet 100 µl of Incubation Buffer in duplicate into wells A1+A2
   • Pipet 100 µl of Standard A in duplicate into wells B1+B2
   • Pipet 100 µl of Standard B in duplicate into wells C1+C2.
   • Pipet 100 µl of Standard C in duplicate into wells D1+D2.
   • Pipet 100 µl of Standard D in duplicate into wells E1+E2.
   • Pipet 100 µl of Standard E in duplicate into wells F1+F2.
4b. Pipet 100 µl of the Low Control in duplicate into wells G1+G2.
   • Pipet 100 µl of the High Control in duplicate into wells H1+H2.
4c. Pipet 100 µl of each diluted sample in duplicate into the subsequent wells.

5. Cover the plate with a plate sealer, place the plate on a plate rotator set at 800–1000 rpm and incubate for 1 hour ± 5 minutes at 18-28°C.

6. Remove and discard the plate sealer. Empty the wells and wash three times using at least 350 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper. Remove any bubbles.

7. Pipet 100 µl of Enzyme Label to all wells.

8. Cover the plate with a plate sealer, place the plate on a plate rotator set at 800–1000 rpm and incubate for 30 minutes ± 5 minutes at 18-28°C.

9. Remove and discard the Plate Sealer. Empty the wells and wash three times using at least 350 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper. Remove any bubbles. Important: Allow the pNPP substrate solution to reach 18-28°C.

10. Pipet 100 µl of the pNPP Substrate Solution to all wells.

11. Cover the plate with a plate sealer, place the plate on a plate rotator set at 800-1000 rpm, protect the plate from direct light and incubate for 30 ± 5 minutes at 18-28°C.

12. Pipet 100 µl of Stop Solution to all wells. Remove any air bubbles with a pipette tip. Proceed to step 13. within 30 minutes.

13. Read the absorbance at 405 nm in a microtiter plate reader.

### IX. RESULTS AND QUALITY CONTROL

#### Standard Curve
1. Record the absorbance at 405 nm for each standard and blank well.
2. Average the duplicate values, subtract the average of the blank wells and record averages (= corrected average absorbance).
3. Plot the absorbance (vertical axis) versus the CIC concentration in µg Eq/ml of the standards (horizontal axis) using lin/log graph paper.
4. Draw the best fitting curve or calculate the standard curve using a spline smoothed fitting algorithm.

#### Samples and Controls
1. Record the absorbance at 405 nm for each sample and control well.
2. Average the duplicate values, subtract the average of the blank wells and record the averages (=corrected average absorbance).
3. Locate the corrected absorbance value of the sample on the vertical axis, draw a horizontal line intersecting the standard curve and read the CIC concentration (µg Eq/ml) from the horizontal axis.
**Typical Data**

These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

<table>
<thead>
<tr>
<th>Conc. (µg Eq/ml)</th>
<th>Abs (OD)</th>
<th>Calc. Conc. (µg Eq/ml)</th>
<th>CV Conc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.123</td>
<td>0.124</td>
<td>0.124</td>
</tr>
<tr>
<td>Blank Avg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard A</td>
<td>50</td>
<td>1.464</td>
<td>50.26</td>
</tr>
<tr>
<td>Standard A Avg.</td>
<td>50</td>
<td>1.454</td>
<td>49.73</td>
</tr>
<tr>
<td></td>
<td>1.459</td>
<td>50.00</td>
<td>0.74</td>
</tr>
<tr>
<td>Standard B</td>
<td>20</td>
<td>0.767</td>
<td>19.81</td>
</tr>
<tr>
<td>Standard B Avg.</td>
<td>20</td>
<td>0.773</td>
<td>20.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.00</td>
<td>1.33</td>
</tr>
<tr>
<td>Standard C</td>
<td>10</td>
<td>0.455</td>
<td>9.938</td>
</tr>
<tr>
<td>Standard C Avg.</td>
<td>10</td>
<td>0.459</td>
<td>10.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.00</td>
<td>0.87</td>
</tr>
<tr>
<td>Standard D</td>
<td>4</td>
<td>0.263</td>
<td>4.00</td>
</tr>
<tr>
<td>Standard D Avg.</td>
<td>4</td>
<td>0.263</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Standard E</td>
<td>1</td>
<td>0.157</td>
<td>1.01</td>
</tr>
<tr>
<td>Standard E Avg.</td>
<td>1</td>
<td>0.157</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>1.42</td>
</tr>
<tr>
<td>Control LOW</td>
<td></td>
<td>0.142</td>
<td>0.82</td>
</tr>
<tr>
<td>Control Low Avg.</td>
<td></td>
<td>0.143</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.83</td>
<td>1.20</td>
</tr>
<tr>
<td>Control HIGH</td>
<td></td>
<td>1.159</td>
<td>30.78</td>
</tr>
<tr>
<td>Control High Avg.</td>
<td></td>
<td>1.161</td>
<td>31.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.09</td>
<td>1.40</td>
</tr>
</tbody>
</table>

**Optical Density (405 nm)**

*Example Only*

![Optical Density Graph](image)
QUALITY CONTROL

A thorough understanding of this package insert is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this package insert. Since there is no control serum for CIC commercially available, we recommend using a positive serum pool for internal quality controls. All controls must fall within established confidence limits. The confidence limits for the Controls are lot-specific and printed on the additional data sheet.

The following data should be recorded for each assay:
- Kit lot number.
- Opening, dilution, reconstitution and storage datas of all kit components.
- Dilution date of the wash buffer.
- Plot of standard curve.
- Concentration value of controls and internal pool sera.
- Absorbance of the highest standard concentration.
- Absorbance of the reagent blank (Incubation Buffer).

X. EXPECTED VALUES

The frequency of CIC in normal human sera or plasma was determined using blood samples from asymptomatic volunteer blood donors (adult men and women at the age of 18 to 70 years). 192 samples were assayed according to the assay procedure and the following results, expressed in µg Equivalent per ml (µg Eq/ml), were obtained:

<table>
<thead>
<tr>
<th></th>
<th>Before Elimination</th>
<th>After Elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>192</td>
<td>182</td>
</tr>
<tr>
<td>Units</td>
<td>µg Eq/ml</td>
<td>µg Eq/ml</td>
</tr>
<tr>
<td>Median Value</td>
<td>1.25</td>
<td>1.19</td>
</tr>
<tr>
<td>Mean Value</td>
<td>1.68</td>
<td>1.50</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.34</td>
<td>0.83</td>
</tr>
<tr>
<td>Lowest Value</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Highest Value</td>
<td>8.9</td>
<td>4.14</td>
</tr>
</tbody>
</table>

Note: These titer ranges should be used as guidelines only. It is recommended that each laboratory establishes its own expected ranges.

Proposed Cut-off Titer
A total of 10 strongly elevated values (> mean+3SD) from the results of the 192 apparently healthy normal blood donors were eliminated in three iterative cycles. This resulted in a theoretical cut-off value (mean + 2 SD) of 3.2 µg Eq/ml.

Borderline and Positive
Values between 3.2 and 5.0 µg Eq/ml should be regarded as borderline results (grey zone) and assayed again. Values above 5.0 µg Eq/ml should be regarded as positive.

General limitations of CIC normal ranges: elevated ranges of CIC are known to occur in up to 5.2% of normal blood donors without any clinical manifestations (true negatives outside the normal range).

XI. TROUBLE SHOOTING

The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues:
- Pipetting, temperature controlling and timing devices.
- Instrument calibration.
- Expiration dates of reagents.
- Storage and incubation conditions.
- pNPP Substrate Solution should be colorless.
- Purity of water.
XII. PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run)
The intra-assay precision was calculated from the results of 20 pairs of values obtained in a single run. The values are presented in µg Eq/ml.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.6</td>
<td>0.71</td>
<td>6.13</td>
</tr>
<tr>
<td>2</td>
<td>19.0</td>
<td>0.70</td>
<td>3.66</td>
</tr>
<tr>
<td>3</td>
<td>19.2</td>
<td>0.38</td>
<td>1.98</td>
</tr>
<tr>
<td>4</td>
<td>22.5</td>
<td>0.59</td>
<td>2.65</td>
</tr>
<tr>
<td>Mean:</td>
<td></td>
<td></td>
<td>3.61</td>
</tr>
</tbody>
</table>

Inter-Assay Precision (Run-to-Run)
The inter-assay precision was calculated from the results of 3 pairs of values obtained in 20 different runs. The values are presented in µg Eq/ml.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum low</td>
<td>9.2</td>
<td>1.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Serum medium</td>
<td>14.6</td>
<td>1.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Serum high</td>
<td>29.4</td>
<td>2.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.3</td>
<td>11.3%</td>
</tr>
</tbody>
</table>

Dilution Linearity/Parallelism
Human serum samples containing a low and a high titer of CIC were diluted (50 times) with Incubation Buffer and subsequently assayed according to the assay procedure. The values are presented in µg Eq/ml.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Obs</th>
<th>Exp</th>
<th>O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum low</td>
<td>1/50 (100%)</td>
<td>5.27</td>
<td>-</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>1/100 (50%)</td>
<td>3.19</td>
<td>2.64</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>1/200 (25%)</td>
<td>1.49</td>
<td>1.32</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>1/400 (12.5%)</td>
<td>0.64</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Serum medium</td>
<td>1/50 (100%)</td>
<td>4.63</td>
<td>-</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1/100 (50%)</td>
<td>2.41</td>
<td>2.47</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1/200 (25%)</td>
<td>1.13</td>
<td>1.23</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>1/400 (12.5%)</td>
<td>0.57</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Serum high</td>
<td>1/50 (100%)</td>
<td>21.84</td>
<td>11.96</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>1/100 (50%)</td>
<td>11.96</td>
<td>10.92</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>1/200 (25%)</td>
<td>5.15</td>
<td>5.46</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>1/400 (12.5%)</td>
<td>2.49</td>
<td>2.73</td>
<td>91</td>
</tr>
</tbody>
</table>

Plasma/Serum comparison

<table>
<thead>
<tr>
<th>Sample Comparison</th>
<th>1-Serum</th>
<th>2- EDTA</th>
<th>3-HEPARIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg Eq/ml)</td>
<td>1.865</td>
<td>1.786</td>
<td>1.809</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>High (µg Eq/ml)</td>
<td>13.682</td>
<td>13.307</td>
<td>10.969</td>
</tr>
<tr>
<td>Low (µg Eq/ml)</td>
<td>0.84</td>
<td>0.89</td>
<td>0.93</td>
</tr>
<tr>
<td>Comparative Quotient</td>
<td>1.2</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.04</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td>SD</td>
<td>0.19</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
Standardization
The standards of the CIC-C1Q EIA Kit were calibrated against the WHO (World Health Organization) reference preparation of aggregated human IgG.

Sensitivity
Twenty duplicates of incubation buffer (reagent blank) were assayed in a single run. Mean and standard deviation were calculated for the absorbance values. The minimum detectable dose of CIC complexes was calculated to be 0.58 µg Eq/ml by adding two standard deviations to the mean absorbance of the reagent blank (incubation buffer) and intersecting this value with the standard curve obtained in the same run.

The functional least detectable dose (FLDD) was calculated to be < 1 µg Eq/ml (cut-off of intra assay CV = 15%).

Hook Effect
No hook effect was observed up to 50,000 µg Eq/ml.

Method of Comparison
A sample comparison (n=64) of this assay and another FDA cleared commercial method showed the following correlation:

<table>
<thead>
<tr>
<th></th>
<th>Relative Sensitivity</th>
<th>Relative Specificity</th>
<th>Relative Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24/25 = 96%</td>
<td>38/39 = 97.4%</td>
<td>62/64 = 96.9%</td>
</tr>
</tbody>
</table>

XIII. LIMITATIONS OF PROCEDURE

- FOR IN VITRO Diagnostic Use Only
- The kit should not be used beyond the expiration date on the kit label.
- The reagents supplied with this kit are optimized to measure CIC in human serum or plasma.
- Repeated freezing and thawing of specimens and of reagents supplied in this kit must be avoided.
- The assay should be rejected if one or both of the two Controls fall outside the confidence limits indicated on the additional Control Sheet.
- If the initial concentration of an unknown sample reads greater than the highest standard (Standard A), the sample should be further diluted with Incubation Buffer and assayed again according to the assay procedure. Results should be multiplied by the appropriate dilution factor.
- Please note sera and plasma may contain interfering substances such as: chelating agents, DNA, anti-C1q, rheumatoid factor, monomeric immunoglobulin.

CIC concentrations should be used as supplementary data available to the physician in developing a diagnosis.

XIV. REFERENCES

**XV. CIC-C1Q PIPetting Protocol**

- **Precoated Microtiter Plate**
  - 2 x wash

- **100 µl Incubation Buffer, Standards, Controls, Plasma or Serum Samples (1:50)**
  - 100 µl
  - 1 hour, 18-28°C on a plate rotator at 800-1000 rpm
  - 3 x wash

- **100 µl Enzyme Label**
  - 100 µl
  - 30 min, 18-28°C on a plate rotator at 800-1000 rpm
  - 3 x wash

- **100 µl pNPP Substrate**
  - 100 µl
  - 30 min, 18-28°C on a plate rotator at 800-1000 rpm

- **100 µl Stop Solution**
  - 100 µl
  - Within 30 min

- **Microtiter Plate Reader at 405 nm**

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