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BIOSZEN MANUAL

With a single Excel file you get flexible views and analyses: filter, normalize, and remove data without creating extra files. You can plot multiple parameters simultaneously and relate them— the app manages it for you.

# **1. Prepare Your Input Files**

**Download Templates**

Click “Reference Platemap File (download)” and “Reference Curves File (download)” to get examples of each template already formatted.

**1.1 Structure of the Platemap-Parameters File (.xlsx)**

**Sheet "Data"**

**Required columns:**

* **Well:** well identifier (e.g., A1, B3). Essential for the Curves plot: links each curve to its original sample.
* **Strain:** strain or group name.
* **Media:** condition or treatment (e.g., Control, Treatment A).
* **BiologicalReplicate:** biological replicate number (1, 2, 3…).
* **TechnicalReplicate:** technical replicate (A, B…); leave blank or “A” if none.
* **One column per parameter** you want to plot (e.g., Viability, Fluorescence).
* **Order:** number defining the display and export order of media. Recommended for consistency in both Strain-specific and Combined plots.

**Sheet "PlotSettings"**

Each row corresponds to a parameter in the "Data" sheet.

**Required columns:**

* **Parameter:** exact column name in "Data."
* **Y\_Max:** initial upper limit of the Y axis (numeric).
* **Interval:** tick interval for Y axis (numeric).
* **Y\_Title:** Y-axis label (text).

**1.2 Structure of the Curves File (.xlsx)**

If using the Curves chart type, you need a file with two sheets:

**Sheet1 – Raw Curve Data**

* First column: **Time** (or the X-axis variable).
* Following columns: values for each curve, named by the source well (e.g., A1, B3). Well names must match the "Well" column in the main file.

**Sheet2 – Axis Configuration**

**Required columns:**

* **X\_Max:** initial maximum for the X axis.
* **Interval\_X:** tick spacing for X axis.
* **Y\_Max:** initial maximum for the Y axis.
* **Interval\_Y:** tick spacing for Y axis.
* **X\_Title:** X-axis label.
* **Y\_Title:** Y-axis label.

# **2. App Interface**

Follow these steps in order:

1. **Load Metadata-Parameters (.xlsx):** select your Excel file with "Data" and "PlotSettings".
2. **Load Curves (.xlsx):** select your Curves file (optional).
3. **Instructions (download):** download this manual in Word.
4. **Reference Platemap File (download):** download the Platemap template.
5. **Reference Curves File (download):** download the Curves template.
6. **Scope:** choose Strain-specific or Combined.
7. **Strain:** dropdown with detected strains (only in Strain-specific).
8. **Chart Type:** choose Boxplot, Bar Chart, Curves, Stacked, or Correlation.
9. **Specific Settings:** appear based on selected chart type:
   * **Stacked:** parameters, order, error bars.
   * **Correlation:** X & Y axes, method, regression line, labels.
   * **Curves:** axis limits, intervals, titles.
   * **Normalization:** toggle "Normalize to control" and select the reference media.
   * **Filters:** adjust Media, Groups, and Replicates based on scope.
   * **Axis Y & Style:** scale, titles, font size, line thickness.
   * **Parameter & Title:** choose parameter, order, manual title.
10. **Downloads:** PNG, Data, Metadata, and Statistical Results.

# **3. Data Processing**

The app computes each BiologicalReplicate as the mean of its TechnicalReplicates. Data are grouped by Strain, Media, and BiologicalReplicate, and averaged for each parameter defined in PlotSettings.

# **4. Scope and Group Selection**

**Scope**

* **Strain-specific:** one chart per strain.
* **Combined:** all "Strain–Media" groups in a single chart.

**Strain-specific**

* **Strain:** dropdown of detected strains.
* **Filter Media:** select/deselect all; checkboxes for each media.
* **Replicates:** choose which BiologicalReplicates to show.
* **Order (csv):** manual order of media on the X axis.

**Combined**

* **Filter Groups:** checkboxes for each "Strain – Media".
* **Show Strain in Labels:** toggle.
* **Replicates:** filter BiologicalReplicates per group.
* **Order (csv):** manual order of groups.

# **5. Choosing Chart Type and Colors**

* **Chart Type:** Boxplot | Bar | Curves (requires Curves file)
* **Color Palette:** Default | Black & White | Viridis | …

# **6. Normalizing Data to Control**

* **Enable Normalization:** toggle "Normalize to control."
* **Reference Media:** select the media to use as baseline (value = 1).

**Requirements:**

* Equal number of BiologicalReplicates across groups.
* Each strain normalizes to its own control, even in Combined scope.

**Behavior:**

* The app divides each BiologicalReplicate by its corresponding control replicate (Replicate 1 vs Replicate 1, etc.).
* Boxplot, Bar, and Correlation charts use normalized values; Curves do not.
* Statistical tests can run on normalized data (deselect control to avoid constant groups).

# **7. Stacked Charts**

Stack multiple parameters in the same column by "Strain–Media."

* **Parameters:** select which to stack.
* **Stack Order:** bottom-to-top list, comma-separated.
* **Scope & Normalization:** same options as other charts.
* **Error Bars:** toggle standard deviation.
* **Interactivity:** parameters remain accessible individually; tests compare same parameters only.

# **8. Scale Settings and Titles**

* **Y\_Max:** top limit for Y axis (0 = use PlotSettings or normalization value).
* **Y\_Interval:** tick spacing for Y axis.
* **Chart Title:** auto-generated if left blank.

# **9. Image Size and Style**

* Width & Height (px).
* Title, Axes, Legend Font Size.
* Axis Line Thickness.

# **10. Statistical Analysis**

In Select Charts → Statistical Analysis, two tabs are available.

**10.1 Normality Tests**

* Shapiro–Wilk (stats::shapiro.test)
* Kolmogorov–Smirnov (stats::ks.test)
* Anderson–Darling (nortest::ad.test)

Click "Run Normality" to get p-values and Yes/No decisions (p > 0.05).

*Note:* On normalized data, deselect the control to avoid zero-variance groups.

**10.2 Significance Tests**

**Global Tests:**

* ANOVA (stats::aov)
* Kruskal–Wallis (stats::kruskal.test)
* Independent t-test (rstatix::t\_test)
* Independent Wilcoxon (rstatix::wilcox\_test)

**Posthoc Options:**

* Tukey (stats::TukeyHSD)
* Bonferroni, Sidak (rstatix::pairwise\_t\_test)
* Dunnett (DescTools::DunnettTest)
* Scheffé, Conover, Nemenyi, DSCF (PMCMRplus)
* Games–Howell (rstatix::games\_howell\_test)

**Modes:**

* All vs. All
* Control vs. All
* Paired

Click "Run Significance" to get comparisons, p-values, Yes/No decisions, and stars.

# **11. Adding Significance Bars**

* **Group 1 & Group 2:** select groups to compare.
* **Label:** e.g., \*, \*\*, n.s.
* Click "Add Bar." Bars stack without removing previous ones.
* "Clear All" removes all bars.
* Adjust line thickness, separation, and label size.

*Note:* Significance bars only appear on Boxplot and Bar charts, not Curves.

# **12. Correlation Plot**

Explore relationships between two parameters with full filtering and customization.

* **Axis Selection:** raw or normalized values.
* **Normalization:** use normalized values if enabled.
* **Method:** Pearson or Spearman; coefficient and p-value displayed.
* **Regression Line:** optional dashed line.
* **Point Labels:** adjustable; ggrepel avoids overlaps.
* **Scope:** Strain-specific or Combined with same filters.
* **Axis Limits & Intervals:** define min, max, and tick spacing.
* **Title:** default "Correlation Y vs X," editable.
* **Download:** high-resolution PNG.

# **13. Downloading Results**

* **PNG (300 dpi).**
* **Data:** detailed and summary tables.
* **Metadata:** current configuration.
* **Statistical Results:** tests performed for all parameters.

# **14. Dynamic Statistical Analysis**

Tests always run on the parameter selected in Boxplot/Bar.

* **Strain-specific:** compares active Media.
* **Combined:** compares "Strain–Media" combinations.
* After changing filters or replicates, click "Run Normality" or "Run Significance" again to update.

# **15. Growth Rates**

In the Growth Rates tab you can calculate and download growth parameters from Excel curve files. Below is its functionality:

**Input Files**

* **Load Growth Curves (.xlsx):** select one or more Excel files. Each file must have a sheet with raw curve data (Time column and well-named value columns).
* **Format Example:** designed for Tecan-generated files; if using another source, the first two columns are ignored and data start from the third column (first well).

| **Well positions** |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Raw data |  |  |  |  |  |  |
|  |  | A1 | A2 | A3 | A4 | … |
| 0 | 37.0 °C | 0.149 | 0.148 | 0.152 | 0.143 | … |
| 1800 | 37.0 °C | 0.147 | 0.145 | 0.147 | 0.141 | … |
| 3600 | 37.0 °C | 0.144 | 0.143 | 0.144 | 0.139 | … |
| … | … | … | … | … | … | … |

*Ensure curves reach at least the specified Max Time (e.g., 48); otherwise, parameter calculation may fail or be incomplete.*

**Required Parameters**

* **Max Time:** upper time limit (e.g., 48).
* **Interval (by):** sampling interval (e.g., 0.5). Same unit as Max Time.

**Buttons**

* **Calculate Parameters:** processes each curve and outputs:
  + **µMax:** maximum growth rate.
  + **max\_percap\_time:** average time in exponential phase.
  + **doub\_time:** doubling time (log(2)/µMax).
  + **lag\_time:** lag phase duration.
  + **ODmax:** maximum OD reached.
  + **max\_time:** time at ODmax.
  + **AUC:** area under the curve.
* **Download Results:** downloads a ZIP containing for each input file:
  + **Curves\_.xlsx:** cleaned curves and axis settings.
  + **Parameters\_.xlsx:** table of calculated parameters.
* **Import to Charts & Stats:** if only one file was loaded, this adds the generated parameters to the Charts & Stats module for plotting with your platemap.

*Important:* Ensure the platemap loaded in the Charts & Stats tab includes in its PlotSettings sheet the names of the parameters you want to plot; otherwise, they won’t appear. Also, leave the Curves data section in Charts & Stats empty so it accepts the cleaned curves from Growth Rates.

**Preview**

The **growthTable** displays all calculated parameters for review before downloading.

**Tips**

* Delete empty well columns before upload to speed up calculations; then sync your platemap to avoid mismatches.
* Always keep the platemap loaded in Charts & Stats, as import requires matching wells to growth data.

**Recommended Workflow**

1. Upload your main and/or curves files.
2. Explore the interactive chart.
3. Adjust scale, colors, and titles.
4. Filter groups and replicates.
5. (Optional) Normalize data.
6. Run Normality and Significance if needed.
7. Download the image or ZIP with everything.

# **Unified File Format for Multiple Plates**

**Platemap-Parameters File**

* Add new plates as additional rows below, without repeating column headers.
* Keep numbering consecutive (e.g., continue from H13) to maintain correspondence.

**Curves File**

* Append new curves to the right of existing ones (applies both to plotting and growth parameter files).
* Don’t repeat column headers; if the first plate ends at H12, start new columns at H13, H14, etc.
* Add as many plates as needed, respecting your experimental design.

Example: Plate 1 with replicates 1 & 2; Plate 2 with replicates 3 & 4. This mapping must reflect in both files to combine measurements correctly.

# Contact Information

For comments, suggestions, or support, contact: [**bioszenf@gmail.com**](mailto:bioszenf@gmail.com)

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