

# Package ‘seahtrue’

November 4, 2024

**Type** Package

**Title** Seahtrue revives XF data for structured data analysis

**Version** 1.1.0

**Description** Seahtrue organizes oxygen consumption and extracellular acidification analysis data from experiments performed on an XF analyzer into structured nested tibbles. This allows for detailed processing of raw data and advanced data visualization and statistics. Seahtrue introduces an open and reproducible way to analyze these XF experiments.

It uses file paths to .xlsx files. These .xlsx files are supplied by the user and are generated by the user in the Wave software from Agilent from the assay result files (.asyr). The .xlsx file contains different sheets of important data for the experiment;

1. Assay Information - Details about how the experiment was set up.
2. Rate Data - Information about the OCR and ECAR rates.
3. Raw Data - The original raw data collected during the experiment.
4. Calibration Data - Data related to calibrating the instrument.

Seahtrue focuses on getting the specific data needed for analysis. Once this data is extracted, it is prepared for calculations through preprocessing. To make sure everything is accurate, both the initial data and the preprocessed data go through thorough checks.

**biocViews** CellBasedAssays, FunctionalPrediction, DataRepresentation, DataImport, CellBiology, Cheminformatics, Metabolomics, MicrotitrePlateAssay, Visualization, QualityControl, BatchEffect, ExperimentalDesign, Preprocessing, GO

**License** Artistic-2.0

**Encoding** UTF-8

**LazyData** false

**Suggests** rmarkdown, knitr, testthat (>= 3.0.0), BiocStyle

**Imports** dplyr (>= 1.1.2), readxl (>= 1.4.1), logger (>= 0.2.2), tidyxl (>= 1.0.8), purrr (>= 0.3.5), tidyr (>= 1.3.0), lubridate (>= 1.8.0), stringr (>= 1.4.1), tibble (>= 3.1.8), validate (>= 1.1.1), rlang (>= 1.0.0), glue (>= 1.6.2), cli (>= 3.4.1), janitor (>= 2.2.0), ggplot2 (>= 3.5.0), RColorBrewer (>=

1.1.3), colorspace ( $\geq 2.1.0$ ), forcats ( $\geq 1.0.0$ ), ggribes ( $\geq 0.5.6$ ), readr ( $\geq 2.1.5$ ), scales ( $\geq 1.3.0$ )

**RoxygenNote** 7.3.1

**Depends** R ( $\geq 4.2.0$ )

**VignetteBuilder** knitr

**URL** <https://vcjdeboer.github.io/seahtrue/>

**BugReports** <https://vcjdeboer.github.io/seahtrue/issues>

**git\_url** <https://git.bioconductor.org/packages/seahtrue>

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**Author** Vincent de Boer [cre, aut] (ORCID:  
<https://orcid.org/0000-0001-9928-1698>),  
 Gerwin Smits [aut],  
 Xiang Zhang [aut]

**Maintainer** Vincent de Boer <vincent.deboer@wur.nl>

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## Description

Seahtrue organizes oxygen consumption and extracellular acidification analysis data from experiments performed on an XF analyzer into structured nested tibbles. This allows for detailed processing of raw data and advanced data visualization and statistics. Seahtrue introduces an open and reproducible way to analyze these XF experiments. It uses file paths to .xlsx files. These .xlsx files are supplied by the user and are generated by the user in the Wave software from Agilent from the assay result files (.asyr). The .xlsx file contains different sheets of important data for the experiment; 1. Assay Information - Details about how the experiment was set up. 2. Rate Data - Information about the OCR and ECAR rates. 3. Raw Data - The original raw data collected during the experiment. 4. Calibration Data - Data related to calibrating the instrument. Seahtrue focuses on getting the specific data needed for analysis. Once this data is extracted, it is prepared for calculations through preprocessing. To make sure everything is accurate, both the initial data and the preprocessed data go through thorough checks.

## Author(s)

**Maintainer:** Vincent de Boer <vincent.deboer@wur.nl> ([ORCID](#))

Authors:

- Gerwin Smits
- Xiang Zhang

## See Also

Useful links:

- <https://vcjdeboer.github.io/seahtrue/>
- Report bugs at <https://vcjdeboer.github.io/seahtrue/issues>

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glue\_xfplates

*Glueing multiple plates from a folder*

---

## Description

This function takes a folder path and on the available .xlsx files the `revive_xfplate()` function is run and output in one nested tibble.

## Usage

```
glue_xfplates(folderpath_seahorse, arg_is_folder)
```

## Arguments

`folderpath_seahorse`

the path to a folder where the .xlsx files are located or a vector of strings pointing to the path of each individual file

`arg_is_folder`

either TRUE or FALSE. When the input is a vector of path strings use FALSE, is it points to a folder use TRUE

**Value**

a nested tibble with all files organized in a row

**Examples**

```
c(
  system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
    package = "seahtrue"
  ),
  system.file("extdata",
    "20191219_SciRep_PBMCs_donor_A.xlsx",
    package = "seahtrue"
  )
) |>
  glue_xfplates(arg_is_folder = FALSE)
```

---

revive\_output\_donor\_A *Dataset PBMC donor A*

---

**Description**

A dataset containing output for the "20191219\_SciRep\_PBMCs\_donor\_A.xlsx" file. This experiment was part of the study that was published in Janssen et al. (2021) Sci rep 11:1662. This is data from PBMCs that were isolated from buffy coats as described in the material and methods section of Janssen et al. (2021). The .xlsx file is the result of a single XF experiment on one plate. This .xlsx file was used as input for the revive\_xfplate() function. The output of that function contains the read, preprocessed, and validated data in the form a a nested tibble. The file '20191219\_SciRep\_PBMCs\_donor\_A.xlsx' can be found in the inst/extdata directory.

**Usage**

```
data(revive_output_donor_A)
```

**Format**

A data frame with 1 row and 7 variables.

**plate\_id** Barcode plate id of the well plate containing the samples

**filepath\_seahorse** Path, and basename to .xlsx file

**date\_run** Date and time when the plate was run

**date\_processed** Date and time this output from revive\_xfplate() was generated

**assay\_info** Meta information from 'Assay Configuration' sheet and 'Calibration' sheet

**injection\_info** Dataframe with information from the 'Operation log' sheet

**raw\_data** Preprocessed raw dataframe from 'Raw' sheet

**rate\_data** Preprocessed rate data from 'Rate' sheet

**validation\_output** A list of all validation information, output, and rules that are used

**Source**

Janssen et al. 2021 Sci Rep 11:1162 <<https://doi.org/10.1038/s41598-021-81217-4>>

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revive_xfplate	<i>Running the read, preprocess and validate</i>
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**Description**

This function takes the Seahorse Wave .xlsx file and computes it through read, validate and preprocess

**Usage**

```
revive_xfplate(filepath_seahorse)
```

**Arguments**

filepath\_seahorse  
Absolute path to the Seahorse Excel file.

**Value**

A preprocessed seahorse dataset is returned as an output. This is a nested tibble with the following 7 columns:

- \* plate\_id = Barcode plate id of the well plate containing the samples
- \* filepath\_seahorse = Path, and basename to .xlsx input file
- \* date\_run = Date and time when the plate was run
- \* date\_processed = Date and time this output from revive\_xfplate() was generated
- \* assay\_info = Meta information from 'Assay Configuration' sheet and 'Calibration' sheet
- \* injection\_info = Dataframe with information from the 'Operation log' sheet
- \* raw\_data = Preprocessed raw dataframe from 'Raw' sheet
- \* rate\_data = Preprocessed rate data from 'Rate' sheet
- # validation\_output = Output of the data checks. including rules

**Examples**

```
revive_xfplate(  
  system.file("extdata",  
    "20191219_SciRep_PBMCs_donor_A.xlsx",  
    package = "seahtrue"  
  )  
)
```

---

`sketch_assimilate_rate`*Combine multiple revived xf plates into one plot for rate data*

---

**Description**

In this plot the OCR or ECAR is plotted per group for each plate in a faceted ggplot

**Usage**

```
sketch_assimilate_rate(my_df, param = "OCR", my_measurements = c(3, 6, 7, 12))
```

**Arguments**

<code>my_df</code>	a tibble generated by <code>glue_xfplates()</code> with for each row representing a single xf experiment
<code>param</code>	either "OCR" or "ECAR"
<code>my_measurements</code>	the measurements that needs to be in the plot. For example, <code>c(3,6,7,12)</code> for a typical mito stress test.

**Value**

a ggplot object

**Examples**

```
suppressMessages(  
  c(  
    system.file("extdata",  
               "20191219_SciRep_PBMCs_donor_A.xlsx",  
               package = "seahtrue"  
    ),  
    system.file("extdata",  
               "20191219_SciRep_PBMCs_donor_A.xlsx",  
               package = "seahtrue"  
    )  
  ) |>  
  glue_xfplates(arg_is_folder = FALSE) |>  
  sketch_assimilate_rate(  
    param = "OCR",  
    my_measurements = c(3, 4, 9, 12)  
  )  
)
```

---

sketch\_assimilate\_raw *Combine multiple revived xfplates into one plot for raw data*

---

## Description

In this plot the O2, pH, or its emission value at the very first measurement point plotted for all wells from all xfplates that are provided to the function.

## Usage

```
sketch_assimilate_raw(my_df, param = "O2_mmHg")
```

## Arguments

my_df	a tibble generated by glue_xfplates() with for each row representing a single xf experiment
param	either "O2_mmHg", "pH", "O2_em_corr" or "pH_em_corr"

## Value

a ggplot object

## Examples

```
suppressMessages(  
  c(  
    system.file("extdata",  
               "20191219_SciRep_PBMCs_donor_A.xlsx",  
               package = "seahtrue"  
    ),  
    system.file("extdata",  
               "20191219_SciRep_PBMCs_donor_A.xlsx",  
               package = "seahtrue"  
    )  
  ) |>  
  glue_xfplates(arg_is_folder = FALSE) |>  
  sketch_assimilate_raw(param = "O2_mmHg")  
)
```

---

sketch_plate	<i>Make an overview of plate layout, with colored groups</i>
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---

### Description

A heatmap style ggplot figure with each well labeled with a color for each group

### Usage

```
sketch_plate(xfplate, reorder_legend = FALSE)
```

### Arguments

xfplate	This the 'raw_data' or the 'rate_data' tibble that is generated by the 'revive_xfplate()' function
reorder_legend	either 'TRUE' or 'FALSE'. When 'TRUE' the groups are ordered based on the number in the character string of the group. It also adds a "__00" after each character string to make the forcats::refactor(group, parse_number(group)) work.

### Value

a ggplot object of a 96 well plate with the group layout

### Examples

```
system.file("extdata",
  "20191219_SciRep_PBMCs_donor_A.xlsx",
  package = "seahtrue"
) |>
  revive_xfplate() |>
  purrr::pluck("raw_data", 1) |>
  sketch_plate(reorder_legend = TRUE)
```

---

sketch_rate	<i>Generate a plot for the rate data</i>
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---

### Description

The sketch\_rate() function uses the rate\_data from the generated output from the revive\_xfplate() function. The injection info is annotated in the plot, using the information from the injections provided in the original experiment. Several options are available to plot either ECAR/OCR or normalize the data with the values from the normalization cells in the .xlsx file.

A number of validations are performed to check whether the data can be plotted and whether the layout of the plot will not be ruined...



**Usage**

```
sketch_rate(
  xf_rate,
  param = "OCR",
  normalize = FALSE,
  normalize_unit = "10000 cells",
  take_group_mean = TRUE,
  reorder_legend = FALSE
)
```

**Arguments**

<code>xf_rate</code>	The 'rate_data' tibble as generated by 'revive_plate'
<code>param</code>	Either "OCR" or "ECAR"
<code>normalize</code>	Either TRUE or FALSE
<code>normalize_unit</code>	any string that will be pasted in the y-axis label when <code>normalize = TRUE</code>
<code>take_group_mean</code>	Either TRUE or FALSE
<code>reorder_legend</code>	Either TRUE or FALSE. When 'TRUE' the groups are ordered based on the number in the character string of the group. It also adds a "__00" after each character string to make the <code>forcats::refactor(group, parse_number(group))</code> work.

**Value**

a ggplot object

**Examples**

```
system.file("extdata",
  "20191219_SciRep_PBMCs_donor_A.xlsx",
  package = "seahtrue"
) |>
  revive_xfplate() |>
  purrr::pluck("rate_data", 1) |>
  sketch_rate(
    param = "OCR",
    reorder_legend = TRUE
  )
```

```
system.file("extdata",
  "20191219_SciRep_PBMCs_donor_A.xlsx",
  package = "seahtrue"
) |>
  revive_xfplate() |>
  purrr::pluck("rate_data", 1) |>
  sketch_rate(
    param = "OCR",
    take_group_mean = FALSE,
    reorder_legend = TRUE
  )
```

```
)  
  
system.file("extdata",  
  "20191219_SciRep_PBMCs_donor_A.xlsx",  
  package = "seahtrue"  
) |>  
  revive_xfplate() |>  
  purrr::pluck("rate_data", 1) |>  
  sketch_rate(  
    param = "ECAR",  
    normalize = TRUE,  
    take_group_mean = TRUE,  
    reorder_legend = TRUE  
  )  
  
system.file("extdata",  
  "20191219_SciRep_PBMCs_donor_A.xlsx",  
  package = "seahtrue"  
) |>  
  revive_xfplate() |>  
  purrr::pluck("rate_data", 1) |>  
  sketch_rate(  
    param = "ECAR",  
    normalize = TRUE,  
    take_group_mean = FALSE,  
    reorder_legend = TRUE  
  )
```

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