

# Package ‘CytobankAPIstats’

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**Type** Package

**Title** Computes Signaling and Population Stats for Cytometry Data on Cytobank using 'CytobankAPI'

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**Description** Tools to process cytometry data from Cytobank into easily usable form for analysis of populations, markers, and signaling using the 'CytobankAPI' package. Learn more about Cytobank at <<https://www.cytobank.org>>. For more information about types of cytometry data that can be analyzed, please see: Bendall, S. C., Simmonds, E. F., Qiu, P., Amir, E. D., Krutzik, P. O., Finck, R.,... Nolan, G. P. (2011) <doi:10.1126/science.1198704> and Adan, Izada, G., Kiraz, Y., Baran, Y., Nalbant, A. (2017). <doi:10.3109/07388551.2015.1128876>.

**License** Artistic-2.0

**Imports** CytobankAPI, shiny, xlsx, shinyFiles, pheatmap

**Suggests** httr, methods, curl, stats, jsonlite

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**NeedsCompilation** no

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analyzedata	<i>Returns a matrix of event counts or raw medians, as specified by inputs. Columns correspond to fcs files and rows to markers in cell types</i>
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### Description

Returns a matrix of event counts or raw medians, as specified by inputs. Columns correspond to fcs files and rows to markers in cell types

### Usage

```
analyzedata(cyto_session, markersofinterest, popsofinterest, exptID, type)
```

### Arguments

cyto\_session - API authentication token for session  
 markersofinterest - Names of channel parameters in Cytobank as list of strings  
 popsofinterest - Names of gates of interest in Cytobank as list of strings  
 exptID - Integer representing an experiment ID on Cytobank account  
 type - boolean with TRUE to analyze events, FALSE to analyze marker intensity statistics

### Value

Returns a data matrix of event counts or raw signal medians, as specified by variable type

### Examples

```
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
markersofinterest<-c("CD3","CD56")
popsofinterest<-c("CD4 T cells","NK cells")
exptID=4
type=TRUE
analyzedata(cyto_session,markersofinterest,popsofinterest,exptID,type)
```

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asinnorm	<i>Computes the arcsinh ratio of a matrix in relation to the specified column</i>
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---

## Description

Computes the arcsinh ratio of a matrix in relation to the specified column

## Usage

```
asinnorm(mat, col, cofactor)
```

## Arguments

mat	- The result of a call to the parsestats function
col	- The index of column to compute ratios against
cofactor	- The cofactor for arcsinh transformation; typically set as 5 for CyTOF

## Value

Returns a matrix with values as the arcsinh ratio of mat normalized to selected column with the desired cofactor

## Examples

```
#Example starting with obtaining data from Cytobank
library(CytobankAPI)
popsofinterest<-c("CD4 T cells", "NK cells")
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
popsinterest<-getpops(popsofinterest,exptno,cyto_session)
fcs<-getfcsfiles(exptno,cyto_session)
type=TRUE
results<-analyzedata(cyto_session,markersofinterest,popsofinterest,exptno,type)
asinnorm(results,col=2,cofactor=5)

#Example with simple data matrix
data<-matrix(1:9,nrow=3,ncol=3,byrow=TRUE)
colnames(data)<-c("Control", "Patient1", "Patient2")
rownames(data)<-c("Marker1", "Marker2", "Marker3")
#Normalizing patient data to control sample with cofactor of 5
asinnorm(data,1,5)
```

---

calcperevent	<i>Calculates percentages of of cell types of interest out of total cell population</i>
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---

**Description**

Calculates percentages of of cell types of interest out of total cell population

**Usage**

```
calcperevent(results)
```

**Arguments**

results      - The result of a call to the parseevents function

**Value**

Returns a matrix with values as percent of first column. Columns correspond to cell types. First column corresponds to the population as a total reference, eg. all live cells run. Rows correspond to fcs files.

**Examples**

```
#Example starting with obtaining data from Cytobank
library(CytobankAPI)
results1<-statistics.event_counts(UserSession, experiment_id, gate_version = 1,
experiment_version, compensation_id,fcs_files, populations = c("Live","NK cells"),
output = "default", timeout = UserSession@long_timeout)
popsinterest<-c("CD4 T cells","NK cells")
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
popsinterest<-getpops(popsinterest,exptno,cyto_session)
fcs<-getfcsfiles(exptno,cyto_session)
results<-parseevents(results1,popsinterest,fcs)
calcperevent(results)

#Example from simple dataset
data<-matrix(9:1,nrow=3,ncol=3,byrow=FALSE)
rownames(data)<-c("Control","Patient1","Patient2")
colnames(data)<-c("Live cells","Cell type 1","Cell type 2")
calcperevent(data)
```

---

CytobankAPIstatsGUI     *Exports processed events and signaling data*

---

**Description**

Exports processed events and signaling data

**Usage**

CytobankAPIstatsGUI()

**Examples**

```
## Not run:  
library(CytobankAPIstats)  
CytobankAPIstatsGUI()  
## End(Not run)
```

---

filterfiles     *Filters a list of fcs files by search terms*

---

**Description**

Filters a list of fcs files by search terms

**Usage**

filterfiles(files, string)

**Arguments**

files            - List of fcs file IDs with FCS file name as names for list  
string           - List of one or more strings of interest as a list to filter samples

**Value**

Returns a list of file IDs matching with names matching string(s)

**Examples**

```
#Example starting with obtaining data from Cytobank
library(CytobankAPI)
exptno<-4
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
files<-getfcsfiles(exptno,cyto_session)
string<-c("patient","IL-1b")
filterfiles(file,string)

#Simple example when list of file names is already available
files<-1:4
names(files)<-c("Pt1 unst.fcs","Pt2 stim.fcs","Ctrl1 unst.fcs","Ctrl2 stim.fcs")
#Filtering file list to contain only unstimulated files
filterfiles(files,"unstimulated")
#Filtering file list to contain only patient files
filterfiles(files,"Pt")
#Filtering file list to contain both unstimulated and patient files
filterfiles(files,c("Pt","unst"))
```

---

get1status

*Filters matrix based on single sample name condition*


---

**Description**

Filters matrix based on single sample name condition

**Usage**

```
get1status(key, results)
```

**Arguments**

key                   - Search string of interest for names  
results               - Results matrix with fcs files corresponding to columns

**Value**

Returns a matrix with columns matching all search keys

**Examples**

```
#Example starting with obtaining data from Cytobank
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
markersofinterest<-c("CD3","CD56")
popsofinterest<-c("CD4 T cells","NK cells")
exptID=4
```

```

type=F
results<-analyzedata(cyto_session,markersofinterest,popsofinterest,exptID,type)
key<-c("Patient","Control")
get1status(key,results)

#Example with simple data matrix
data<-matrix(1:18,nrow=3,ncol=6,byrow=TRUE)
colnames(data)<-c("Ctrl1 unst","Pt1 unst","Pt3 unst","Ctrl1 stim","Pt1 stim","Pt3 stim")
rownames(data)<-c("Marker1","Marker2","Marker3")
#Getting all patient samples
get1status("Pt",data)
#Getting all patient and stimulated samples
get1status(c("Pt","stim"),data)

```

---

get2status	<i>Filters matrix columns based on two conditions per file, e.g. patient status, stimulation, time points, etc.</i>
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---

## Description

Filters matrix columns based on two conditions per file, e.g. patient status, stimulation, time points, etc.

## Usage

```
get2status(key1, key2, results)
```

## Arguments

key1	- Search string of interest for names
key2	- Search string of interest for names
results	- Results matrix with fcs files corresponding to columns

## Value

Returns a list of IDs with names matching search both search strings with names being the description of these features

## Examples

```

#Example starting with obtaining data from Cytobank
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
markersofinterest<-c("CD3","CD56")
popsofinterest<-c("CD4 T cells","NK cells")
exptID=4
type=F

```

```
results<-analyzedata(cyto_session,markersofinterest,popsofinterest,exptID,type)
key1<-"Patient"
key2<-"Untreated"
get2status(key1,key2,results)

#Example with simple data matrix
data<-matrix(1:18,nrow=3,ncol=6,byrow=TRUE)
colnames(data)<-c("Ctrl1 unst","Pt1 unst","Pt3 unst","Ctrl1 stim","Pt1 stim","Pt3 stim")
rownames(data)<-c("Marker1","Marker2","Marker3")
#Getting all stimulated patient samples
get2status(c("Pt","Ctrl"),"stim", data)
#Getting all stimulated patient and control samples
get2status(c("Pt","Ctrl"),"stim", data)
```

---

getfcsfiles

*Gets fcs ID numbers and sample names from a given experiment*

---

## Description

Gets fcs ID numbers and sample names from a given experiment

## Usage

```
getfcsfiles(exptno, cyto_session)
```

## Arguments

exptno           - Integer representing an experiment ID on Cytobank account  
cyto\_session    - API authentication token for session

## Value

Returns a list of fcs file IDs with names of fcs files as names of list

## Examples

```
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
getfcsfiles(exptno,cyto_session)
```



---

getmarkers	<i>Gets appropriate marker IDs for channels of interest</i>
------------	---

---

**Description**

Gets appropriate marker IDs for channels of interest

**Usage**

```
getmarkers(markersofinterest, exptno, cyto_session)
```

**Arguments**

markersofinterest - Names of channel parameters in Cytobank as list of strings  
exptno - Integer representing an experiment ID on Cytobank account  
cyto\_session - API authentication token for session

**Value**

Returns a list of IDs for markers of interest with names of markers as names of list

**Examples**

```
library(CytobankAPI)
markersofinterest<-c("CD3", "CD56")
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
getmarkers(markersofinterest,exptno,cyto_session)
```

---

getnewind	<i>Rearranges signaling results matrix with rows in the desired order as outputs</i>
-----------	--

---

**Description**

Rearranges signaling results matrix with rows in the desired order as outputs

**Usage**

```
getnewind(fixlabels, results)
```

**Arguments**

fixlabels - List of strings with desired order of labels  
results - Output of call to parsestats

**Value**

- Returns a matrix with rows organized in desired order specified by fixlabels

**Examples**

```
#Example starting with obtaining data from Cytobank
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
markersofinterest<-c("CD3","CD56")
popsofinterest<-c("CD4 T cells","NK cells")
exptID=4
type=F
results<-analyzedata(cyto_session,markersofinterest,popsofinterest,exptID,type)
fixlabels<-c("CD4 T cells CD56","NK cells CD56","CD4 T cells CD3","NK cells CD3")
getnewwind(fixlabels,results)

#Example with simple matrix
data<-matrix(1:8,nrow=4,ncol=2,byrow=TRUE)
colnames(data)<-c("Control","Patient")
rownames(data)<-c("NK cells CD3","CD4 T cells CD3","CD4 T cells CD56","NK cells CD56")
fixlabels<-c("CD4 T cells CD56","NK cells CD56","CD4 T cells CD3","NK cells CD3")
getnewwind(fixlabels,data)
```

---

getpops

*Gets appropriate gate set IDs for populations of interest*

---

**Description**

Gets appropriate gate set IDs for populations of interest

**Usage**

```
getpops(popsofinterest, exptno, cyto_session)
```

**Arguments**

popsofinterest - Names of gates of interest in Cytobank as list of strings  
 exptno - Integer representing an experiment ID on Cytobank account  
 cyto\_session - API authentication token for session

**Value**

Returns a list of gateSetIDs for populations of interest with names of populations as names of list

**Examples**

```
library(CytobankAPI)
popsofinterest<-c("CD4 T cells","NK cells")
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
getpops(popsofinterest,exptno,cyto_session)
```

---

getrawsignals	<i>Computes the untransformed medians for cellular markers in populations of interest</i>
---------------	---

---

**Description**

Computes the untransformed medians for cellular markers in populations of interest

**Usage**

```
getrawsignals(cyto_session, markersofinterest, popsofinterest, exptID,
  markerorder, stimterms, ptterms)
```

**Arguments**

cyto\_session - API authentication token for session  
 markersofinterest - List of strings of markers of interest, corresponding to names in Cytobank  
 popsofinterest - List of strings of populations of interest to calculate statistics  
 exptID - Integer representing an experiment ID on Cytobank account  
 markerorder - A list of strings corresponding to the desired marker order  
 stimterms - A list of desired stimulation conditions to analyze in matrix  
 ptterms - A list of desired sample conditions to analyze in matrix

**Value**

- Returns matrix of untransformed medians for cellular markers in populations of interest

**Examples**

```
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
markersofinterest<-c("CD3","CD56")
popsofinterest<-c("CD4 T cells","NK cells")
exptID=4
markerorder<-c("CD4 T cells CD56","NK cells CD56","CD4 T cells CD3","NK cells CD3")
stimterms<-c("Unstim","IL-15")
ptterm<-c("Pt","Ctrl")
```

```
getrawsignals(cyto_session,markersofinterest,popsinterest,exptID,markerorder,stimterms,
ptterms)
```

---

parseevents	<i>Modifies the list obtained from a call to statistics.events to a matrix with rows corresponding to fcs files and columns corresponding to the population types</i>
-------------	---

---

### Description

Modifies the list obtained from a call to statistics.events to a matrix with rows corresponding to fcs files and columns corresponding to the population types

### Usage

```
parseevents(results, popsinterest, fcs)
```

### Arguments

results	- The results of a call to statistics.events function
popsinterest	- List of gateSetID numbers for populations of interest with descriptions as names
fcs	- List of fcs file IDs of interest with description of FCS files as names

### Value

Returns a matrix of event counts with rows corresponding to fcs files and columns corresponding to populations of interest

### Examples

```
library(CytobankAPI)

cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
popsinterest1<-c("CD4 T cells","NK cells")
popsinterest<-getpops(popsinterest1,exptno,cyto_session)
fcs<-getfcsfiles(exptno,cyto_session)
results<-statistics.event_counts(cyto_session, exptno, gate_version = 1,
compensation_id=1,fcs_files=fcs,populations = popsinterest,output = "default",
timeout = UserSession@long_timeout)
parseevents(results,popsinterest,fcs)
```

---

parsestats	<i>Takes the results of a call to statistics.general and returns a matrix of raw medians with columns corresponding to fcs files and rows to molecules of interest in different cell types</i>
------------	--

---

## Description

Takes the results of a call to statistics.general and returns a matrix of raw medians with columns corresponding to fcs files and rows to molecules of interest in different cell types

## Usage

```
parsestats(results, popsinterest, fcs, markersofinterest)
```

## Arguments

results           - The results of a call to statistics.general function  
popsinterest      - List of gateSetID numbers for populations of interest with descriptions as name  
fcs               - List of fcs file IDs of interest with description of FCS file names as names  
markersofinterest  
                  - List of ID numbers for markers of interest with descriptions as name

## Value

Returns a matrix of median signaling intensities with columns corresponding to fcs files and rows corresponding to markers of interest in cell types of interest

## Examples

```
library(CytobankAPI)

cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
popsofinterest1<-c("CD4 T cells", "NK cells")
popsinterest<-getpops(popsofinterest1, exptno, cyto_session)
fcs<-getfcsfiles(exptno, cyto_session)
markersofinterest1<-c("CD3", "CD56")
markersofinterest<-getmarkers(markersofinterest1, exptno, cyto_session)
results<-statistics.general(UserSession=cyto_session, experiment_id=2, gate_version = -1,
compensation_id=1, fcs_files=fcs, populations = popsinterest,
output = "default", timeout = UserSession@long_timeout)
parsestats(results, popsinterest, fcs, markersofinterest)
```

---

parsestatsmean	<i>Takes the results of a call to statistics.general and returns a matrix of raw means with columns corresponding to fcs files and rows to molecules of interest in different cell types</i>
----------------	--

---

### Description

Takes the results of a call to statistics.general and returns a matrix of raw means with columns corresponding to fcs files and rows to molecules of interest in different cell types

### Usage

```
parsestatsmean(results, popsinterest, fcs, markersofinterest)
```

### Arguments

results	- The results of a call to statistics.general function
popsinterest	- List of gateSetID numbers for populations of interest with descriptions as name
fcs	- List of fcs file IDs of interest with description of fcs file names as names
markersofinterest	- List of ID numbers for markers of interest with descriptions as name

### Value

Returns a matrix of mean signaling intensities with columns corresponding to fcs files and rows corresponding to markers of interest in cell types of interest

### Examples

```
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
exptno<-2
popsinterest1<-c("CD4 T cells", "NK cells")
popsinterest<-getpops(popsinterest1, exptno, cyto_session)
fcs<-getfcsfiles(exptno, cyto_session)
markersinterest1<-c("CD3", "CD56")
markersinterest<-getmarkers(markersinterest1, exptno, cyto_session)
results<-statistics.general(UserSession=cyto_session, experiment_id=2, gate_version = -1,
compensation_id=1, fcs_files=fcs, populations = popsinterest,
output = "default", timeout = UserSession@long_timeout)
parsestatsmean(results, popsinterest, fcs, markersofinterest)
```

---

percentevent	<i>Calculates the percentage of cell populations given an experiment</i>
--------------	--

---

**Description**

Calculates the percentage of cell populations given an experiment

**Usage**

```
percentevent(cyto_session, markersofinterest, popsofinterest, exptID, grouping,
specimennames, means)
```

**Arguments**

cyto\_session - API authentication token for session  
markersofinterest - List of names of channel parameters in Cytobank  
popsofinterest - List of populations of interest to calculate percentages with reference population for percentages listed first  
exptID - Integer representing an experiment ID on Cytobank account  
grouping - A list of indices corresponding to samples from the same donor ex list(c(1,2),c(3,4,5)) if rows 1 and 2 are from pt1,3,4,5 are from pt2, etc.  
specimennames - List of specimen names as strings; needs to be same length as number of groupings  
means - A boolean if mean =TRUE, a mean for all groups in the variable group is calculated, otherwise individual means are returned.

**Value**

- Returns either the percentage or mean percentage per specimen of each cell type, as specified by mean parameter

**Examples**

```
library(CytobankAPI)
cyto_session <- authenticate(site="premium", username="myusername", password="mypassword")
markersofinterest<-c("CD3", "CD56")
popsofinterest<-c("CD4 T cells", "NK cells")
exptID=4
grouping<-list(c(1,2), c(3,4,5), c(6,7))
specimennames<-c("Patient1", "Patient2", "Control1")
means=T
percentevent(cyto_session, markersofinterest, popsofinterest, exptID, grouping, specimennames, means)
```

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