**difftools – Make iPSC culture easier**

**mkmed -- Make culture media for differentiation of iPSCs into DaNs**

**SYNOPSIS**

mkmed -d -v [OPTION]...

mkmed --help

mkmed --ko

mkmed --mtesr

mkmed --nb

mkmed --nnb

**DESCRIPTION**

mkmed prints the composition of your culture medium to standard output, provided the day of iPSC differentiation (Kriks protocol) and the required volume. If requested, medium composition also includes ROCKi and AntiAnti. Alternatively, mkmed can be used to print the recipe of basal media (mTeSR, KO DMEM KSR, NNB and NB).

The following options are available:

-d Numerical indicating the day of differentiation protocol. Valid values include any positive integer, including 0.

-v Numerical indicating the required volume of culture medium in milliliter. Valid values include any positive double, excluding 0.

--split Logical indicating if ROCKi should be included. Valid values include TRUE, T, FALSE, and F. Note that --split = TRUE is not applied is -d > 20.

--antianti Logical indicating if Anti-Anti should be included. Valid values include TRUE, T, FALSE, and F.

--mtesr Print the recipe of mTeSR.

--ko Print the recipe of KO DMEM KSR.

--nnb Print the recipe of NNB.

--nb Print the recipe of NB.

--help Show this screen.

**EXAMPLES**

*# Get help on how to use mkmed:*

mkmed --help

*# Get the recipe of mTeSR:*

mkmed --mtesr

*# Get the composition of 25ml of day 0 medium (by default, ROCKi and AntiAnti are not included):*

mkmed -d 0 -v 25

*# Get the composition of 130ml of day 10 medium, with ROCKi included:*

mkmed -d 10 -v 130 --split TRUE

*# Get the composition of 200ml of day 22 medium:*

mkmed -d 22 -v 200

*# Get the composition of 200ml of day 45 medium, with AntiAnti included:*

mkmed -d 45 -v 200 --antianti T

**diffd -- Calculate days or dates of differentiation of iPSCs into DaNs**

**SYNOPSIS**

diffd -rdate -rday [OPTION]...

diffd --help

diffd --TSR

**DESCRIPTION**

diffd prints the value of a target differentiation day or date to standard output, provided a reference date and a reference day. If requested, a differentiation calendar is generated and written to file.

The following options are available:

-rdate Reference date of differentiation matching the reference day. Valid values are in the form YYYY-MM-DD or YYYY.MM.DD. Alternatively, the string "today" can be provided and will be converted into the system's idea of the current date.

-rday Reference day of differentiation matching the reference date. Valid values include any integer.

-tdate Target date for which a differentiation day is requested. Valid values are in the form YYYY-MM-DD or YYYY.MM.DD. Alternatively, the string "today" can be provided and will be converted into the system's idea of the current date.

-tday Target day for which a differentiation date is requested. Valid values include any integer.

--exp Logical indicating if an expansion phase should be included. Valid values include TRUE, T, FALSE, and F. Default is TRUE.

--expdur Numerical indicating the duration of the expansion phase in days. Valid values include any positive integer. Default is 21.

--cal Logical indicating if a differentiation calendar should be printed to standard output. Valid values include TRUE, T, FALSE, and F. Default is FALSE.

--write Absolute or relative path indicating the directory where to write the differentiation calendar. The path must lead to an existing directory. It cannot be used in combination with --cal = FALSE.

--help Show this screen.

--TSR Try it.

**EXAMPLES**

*# Get help on how to use diffd:*

diffd --help

*# Get the date of day 60 given that day 10+17 is on 2022.02.14:*

diffd -rdate 2022.02.14 -rday 10+17 -tday 60

*# Get the date of day 45 given that day -2 is on 2022.02.14 and the expansion phase lasts 23 days:*

diffd -rdate 2022.02.14 -rday -2 -tday 45 --expdur 23

*# Get the differentiation day on 2022.02.18 given that day 61 is on 2022.04.10 without expansion:*

diffd -rdate 2022.04.10 -rday 61 -tdate 2022.02.18 --exp F

*# Get the differentiation day on 2022.05.01 given that today is day 29, and print the calendar:*

diffd -rdate today -rday 29 -tdate 2022.05.01 --cal T

*# Get the date of day 70 given that day 0 is on 2022.05.10, and write the calendar in Desktop folder:*

diffd -rdate 2022.05.10 -rday 0 -tday 70 --write ~/Desktop

**difftools installation**

mkmed and diffd are programs written in R and run from a command line interface which interprets UNIX commands. So, to use them on your computer you need to install the R software and, if using Windows, a UNIX terminal.

In case you already use UNIX terminal, just add the path to the mkmed and diffd files (and the path to Rscript if necessary) to your PATH variable.

If you are not experienced with a UNIX terminal, use difftools\_installer to set up mkmed and diffd on your system (instructions below).

Windows:

1. Install R: <https://cran.r-project.org/bin/windows/base/>

*If you want difftools\_installer to set up mkmed and diffd on your system for you, make sure to install R in /c/Program Files/*

2. Install Git Bash (UNIX terminal): <https://git-scm.com/downloads>

*You don’t need to change the installation default settings. Pin Git Bash to taskbar for easy access*

3. Put the difftools folder in your Desktop

*Note that difftools\_installer can set up mkmed and diffd on your system only if your Desktop folder is in /c/Users/YOUR\_USERNAME/*

4. Open Git Bash and run the following command:

~/Desktop/difftools/difftools\_installer Windows

*If the command returns a message indicating that “difftools setup is complete”, you are good to go. Note that the mkmed and diffd files have moved from your Desktop to a bin folder in your home directory*

5. Quit git bash (important), relaunch it, and type the following command to get started:

mkmed --help

*If you get a warning message stating that LC\_CTYPE setting failed during startup, right-clic in the git bash windows, select “Options…” > “Text”, change “Locale” to “C” and “Character set” to “Default”, clic “Apply” and then “Save”. Close, git bash, relaunch it, try again mkmed --help*

MacOS:

1. Install R: <https://cran.r-project.org/bin/macosx/>

2. Put the difftools folder in your Desktop

3. Open the Terminal application and run the following command:

~/Desktop/difftools/difftools\_installer macOS

*If the command returns a message indicating that “difftools setup is complete”, you are good to go. Note that the mkmed and diffd files have moved from your Desktop to a bin folder in your home directory. Keep Terminal in dock for easy access*

4. Quit Terminal (important), relaunch it, and type the following command to get started:

mkmed --help