

ModFit LT 3.2

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Reference

Website: www.VSH.com

Manual: ModFit LT

Introduction

The most recent version of ModFit installed is 3.2

Major improvements:

- Version 3.1 reads Diva data FCS3.0, no more need for transformation in FCS2.0 format
- Lin log converter for all parameters

This software can on the basis of a model from the distribution:

1. Calculate percentage G_0G_1 vs. S vs. G_2M
2. Calculate the Ploidy of the cell population
3. Calculate the Apoptosis grade of the cell population
4. Calculate the generations within a cell population

The program is installed on Ana1,

1 Calculate percentage G_0G_1 vs S vs G_2M

Preferences:

1. <start/all programs/verity/Modfit LT3.0> (the exe has not been upgraded to 3.1)
2. password: Omega
3. <Edit/autoanalysis settings>
4. AutoDebris: tagged enable
5. AutoAggregates: tagged enable
6. Apoptosis: not tagged enable
7. Linearity: 2.00
8. Autolinearity: not tagged
9. Internal standards: 0
10. External reference standard: -1.00 (irrelevant)
11. <OK>

Auto analysis

1. Load file of interest: File/open/data file
2. Select the parameter for DNA analysis. For PI: < PerCP Cy-5> <OK>

3. Define gates: if desired create gates on preferred plots and select the ones of interest (The program detect debris and aggregates, gating is not necessary) <OK>
4. Click “Auto” icon on the toolbar
5. Processed sample appears on screen
6. Click “diag” icon to check the quality of the analysis.
7. Add experiment name, Tools/keywords select <experiment name> <add> <OK>
8. Add diagnostics to workspace Tools/Fit diagnostics
9. Save or print

Manual analysis

1. Load file of interest: File/open/data file
2. Select the parameter for DNA analysis. For PI: < PerCP Cy-5> <OK>
3. Define gates: if desired create gates on preferred plots and select the ones of interest (The program detect debris and aggregates, gating is not necessary) <OK>
4. Click “Mod” icon on the toolbar
5. Click on “Range” icon and place the line marker “Dip G1” over the first peak over the peak and “Dip G2” over the 2nd peak
6. Click on “Fit” icon
7. Processed sample appears on screen
8. Click “diag” icon to check the quality of the analysis
9. Save or print

Diagnosis interpretation

%CV

The Coefficient of Variation is the standard deviation divided by the mean. The lower the CV is, the higher is the resolution of your populations and the more reliable your analysis.

CV <3% is good (Check)

CV 3-6% is fair (Check)

CV >6% is poor (Check)

Cell number

Modfit takes a minimum of 10 000 cells (no debris or aggregates included) as a cut off for good analysis. This cell number originates from a consensus meeting of DNA flow cytometry analysis in oncology

Cell number >10 000 is good (Check)

Cell number < 10 000 is poor (Check)

Average cells per channel

This is the total amount of cells in cycle divided by the number of channels occupied by the ranged from G1 to G2. For an optimal model more than 100 cells per channel are required.

Average cells per channel < 100 is poor (Check)

Average cells per channel > 100 is good (Check)

Aneuploid fraction

At least 15% of the cells in the sample should be tumor cells

%B.A.D.

This means the percentage of events in between G1 and G2 caused by Background, Aggregates of Debris Should be less than 20%

RCS

This stands for Reduced Chi Square, a value to evaluate the modeling

RCS < 3 is good

RCS 3-5 is fair

RCS >5 is poor

Model type

Check to model type, it should be 1DA0n_DSF

1 - The number of cycles (max 5)

D- Auto debris detection on

A – Auto aggregates detection is on

0 – Auto apoptosis detection is off

D – fist peak is diploid

S – S phase detection is on

F – Floating G2 position (quick model editor, tag box on cycle1 G2M)

Report improvements

1-Add experiment and tube name tot the report

Click the report button and the tools toolbox becomes available

Click on keywords [...]

Search and highlight <EXPERIMENT NAME> in the keyword list. Click <Add>

Search and highlight <TUBE NAME> in the keyword list. Click <Add>

Click <OK>

Click <Fit> button and the two names appear on the worksheet

2-Add diagnostics

Popup menu Tools

<Fit diagnostics>

3-Add G1/G2 Area ratio

Click the report button and the tools toolbox becomes available

Click on [Σ]

Click <Add>

Type in current value

“Ratio G1/G2: “;A[5,0]/A[6,0]

Click <OK>

Click <Fit> button and equation appear on the at the bottom of the report list.

Components distribution (I guess), necessary for calculations.

1 = debris

2 = standard

3 = aggregates

4 = Apoptosis

5 = G1 cycle 1

6 = G2 cycle 1

7 = S cycle 1

8 = G1 cycle 2

9 = G2 cycle 2

10 = S cycle 2

Automatic peak search adjustment

Load sample

Mod and Fit

In case the black triangles are not under your peaks you should adapt the parameters for peak search

<Edit> <Peak Finder settings> <Show...>

Place the mouse under an undefined peak and click

Check which parameters have a **pass** and which **low**

Adjust the **low** parameters to **pass**