OLS® OMNI Life Science

CASY TT – Measuring Mammalian Cells

Always use freshly filtered CASYton (Dispenser with correct disk filter attached)!

A. Preparing a Measurement

Activate Test Setup (*) and measure CASYton:

→ Result:

< 100 counts/ml; go to „B."

> 100 counts/ml:

Execute one [**CLEAN**] with CASYton

Repeat measurement with fresh CASYton until counts/ml <100 – no further [**CLEAN**]!!

B. Performing a Measurement Series

1. Select the corresponding cell-specific setup
2. Dispense 10ml CASYton and add your sample, typically 10 - 200µl (**)
3. Mix: slowly tilt closed CASYcup 3x up and down, never shake!!
4. Measure; if counts are:

   > 1 000: go to „B.5" or „C." (any counts between 2.000 and 10.000 are perfect)

   < 1 000 or message [**Concentration too high**]: adjust dilution, store new factor in setup and repeat measurement
5. Next sample:
   - Same cell type and similar cell number (+/-50%): measure one after the other, do not [**CLEAN**]
   - Other cell type or largely different cell number (> 50%): dip capillary shaft into CASYcup with fresh CASYton, wait 5 sec and run next measurement, do not [**CLEAN**]

C. End of Measurements (days end or longer break)

1. Perform 1x [**CLEAN**] with CASYton
2. Put a CASYcup with freshly filtered CASYton to the capillary

D. Error Messages: [Timeout] and [Large Air Bubble detected]

1. [**Timeout**], and air bubble not visible: simply repeat measurement
2. Air bubble stands still in rising tube or error repeats: terminate measurement, replace sample by a CASYcup with CASYton and execute 1x [**CLEAN**]
3. Repeat measurement of sample

E. Weekly Cleaning

Advice: skip the triple measurements mentioned in the main manual

1. Terminate measurement as outlined under „C."
2. Put one CASYcup with 10ml distilled water under capillary and to CASYton intake tube; 3x [**CLEAN**]
3. Repeat step 2 with cups with CASYclean; let it stand for at least 3 hours, or overnight
4. Repeat step 2 with dist. Water (2x) and then rinse with CASYton (1x)
5. Put storage container with CASYton to the intake tube and continue as shown under „A."

(*) Settings background-Cap150.SET

- X-Axis 0 to 50µm
- Evaluation cursor 0 to 50µm
- Sample volume 1x 400µl
- Dilution factor 1 (one)
- All calculations: OFF

(**)Typical Dilution Factors

Cell concentration	Dilution	Dilution Factor
1x 10E5 to 5x 10E5 cells/ml:	10ml CASYton + 200µl Sample, Dilution Factor	51
2x 10E5 to 1x 10E6 cells/ml:	10ml CASYton + 100µl Sample, Dilution Factor	101
4x 10E5 to 2x 10E6 cells/ml:	10ml CASYton + 50µl Sample, Dilution Factor	201
1x 10E6 to 5x 10E6 cells/ml:	10ml CASYton + 20µl Sample, Dilution Factor	501
2x 10E6 to 1x 10E7 cells/ml:	10ml CASYton + 10µl Sample, Dilution Factor	1001

F. Generating a Cell-specific Setup

1. Copy any existing setup and load
Select if possible an existing similar setup, recommended basic parameters are: 3x 400µl, X-Axis 50µm, %Calculation [% Via] and store Setup at a free setup position.

2. Determining Viability Differentiation
You need: a highly vital sample of your new cell type (> 90% Viability). Cell lines should be in exponential growth phase.

i. Measure „viable“ sample according to „B.“; adjust dilution to achieve > 4000 counts(**)
ii. Prepare „DEAD“ sample: mix same sample volume with min. 4-fold excess of CASYblue (10 - 100µl sample + 400µl CASYblue), then incubate for 2min at ambient temperature, then add 10ml CASYton to terminate killing process
iii. Mix viable and dead samples: to about 7 ml of viable add 3-4 ml dead sample, measure mixture (see „B.“)
iv. Move left evaluation cursor (CL) to the lowest count between live and dead cells peak
v. Move left normalization cursor (NL) to the lowest count between dead cells and debris peak
vi. Leave both right cursors (CR) and (NR) at the right end of the scale
vii. Edit Setup to store the cursor settings permanently

3. X-Axis scaling factor
Select right end of scale (upper end) alike to meet ca. 4-fold of the live cells peak diameter [PeakDia].

4. Aggregation correction (optional)
For suspension cells or mixes with different diameter cells -> [Off].
Trypsinized Cells:

- Viability >80% and Counts >2000 → select [Automatic] or [Manual]
- Viability <80% or Counts <2000 → always select [Manual]

Settings:
[Manual]: Aggregation calculation with user defined peak volume, use peak volume average of at least 5 measurements of viable cells with viability >90% and Counts >2000 and store in Setup.
[Automatic]: Aggregation calculation using the measured peak volume of actual sample.

G. Setting System out of Operation, no use for > 2 weeks
1. Follow weekly cleaning routine as under „E.“ until step 3
2. Put a CASYcup with 10ml dist. Water to capillary and the CASYton intake tube;
   3x [CLEAN]
3. Repeat step 2
4. Replace CASYton storage bottle by empty CASYcup; put another empty CASYcup under capillary
5. Run 2x [Dry Liquid System] (can be found under [MENU -> Service]
6. Empty and dry waste and CASYton storage-container and put back in place
7. Remove outer electrode and store in the protective box, and finally apply capillary protector
8. When starting system up again, start with „E.“ (Weekly Cleaning) from step 2 onwards

All about CASY - Troubleshooting, FAQs, Videos, Manuals and Software for your CASY: casy-support.com
Order Consumables, Service and Support: www.ols-bio.de/casy
You need urgent support or you have any question? Write an Email or call us at:
Email: info@ols-bio.de | Telephone: 0421-276-1690