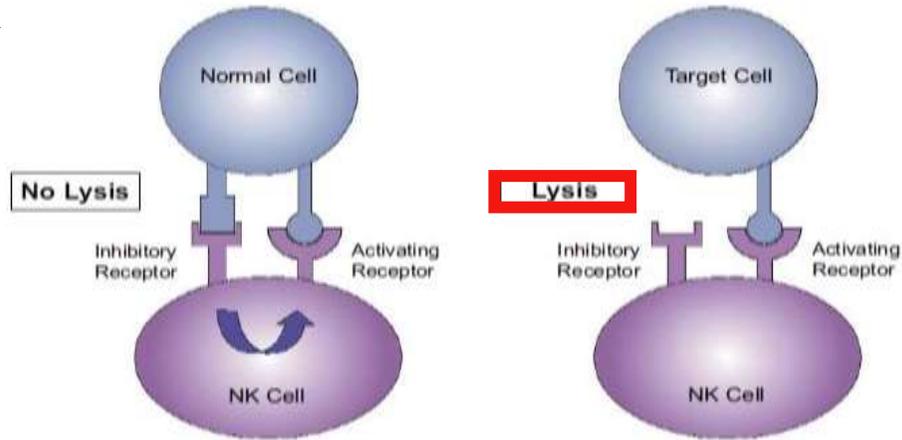


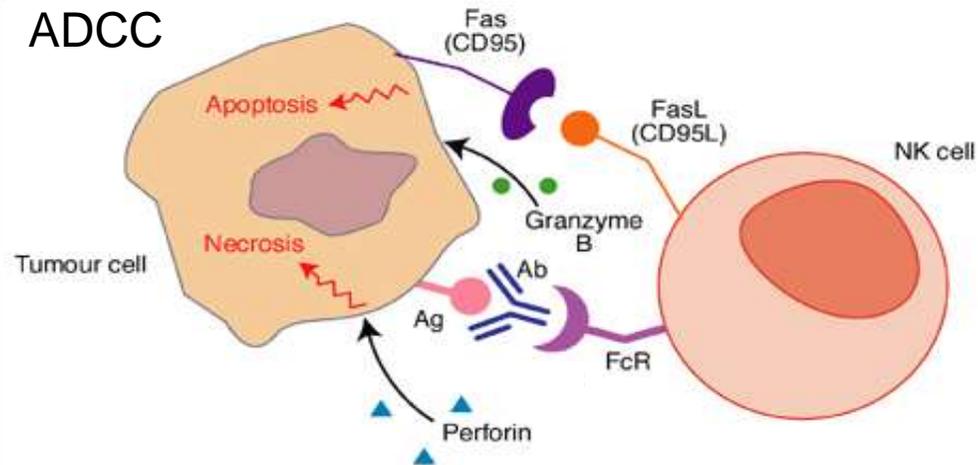
NK/ADCC Target cell Visualization Assay (TVA™)

Background

NK



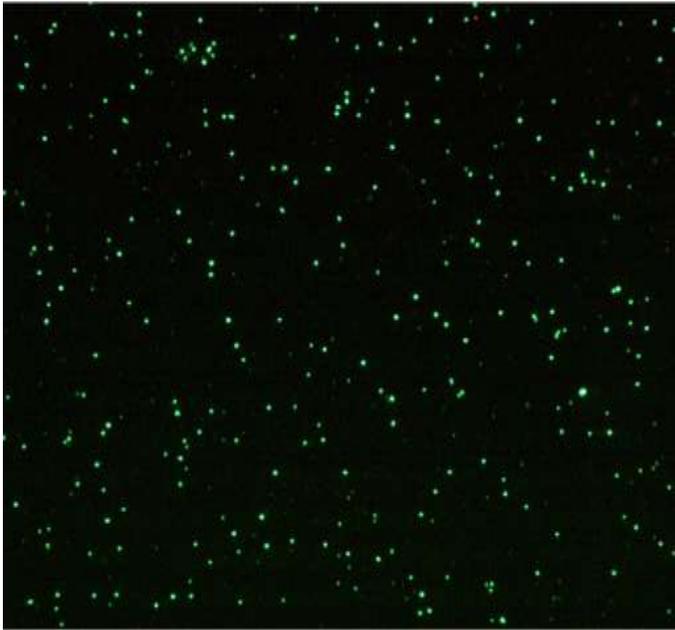
ADCC



Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

Foundation of NK-TVA™ Assay:

Fluorescently-stained target cells lose the dye after they die



Live Target Cells



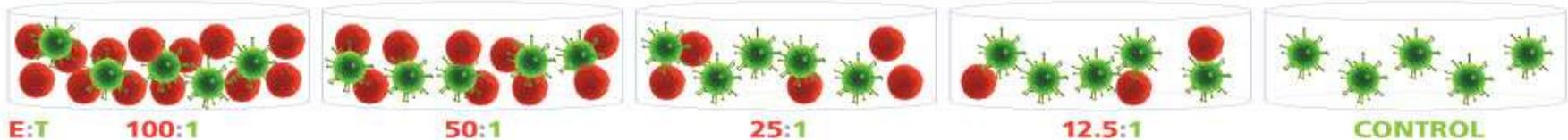
Dead Target Cells

Tumor cells imaged before (left) and after (right) undergoing apoptosis

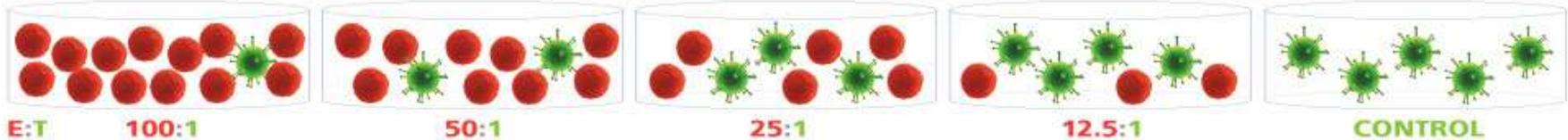
Measuring NK activity in vitro

CTL NK Target cell Visualization Assay (TVA™)

At 0 hours



After 3 hours



● PBMC (NK Cells – E) added at different E:T ratios

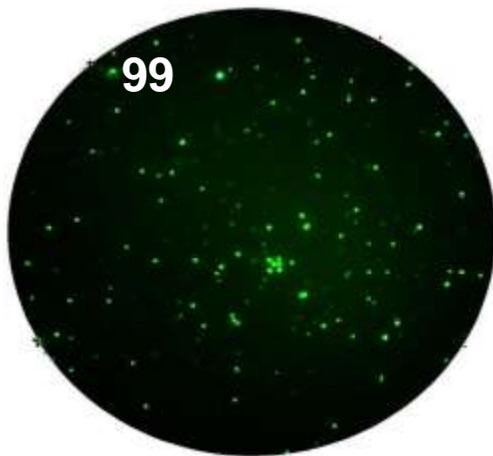
★ Stained Target Cells (T) plated at constant numbers

NK activity monitored by measuring the number of viable target cells using high-throughput, single-cell imaging.

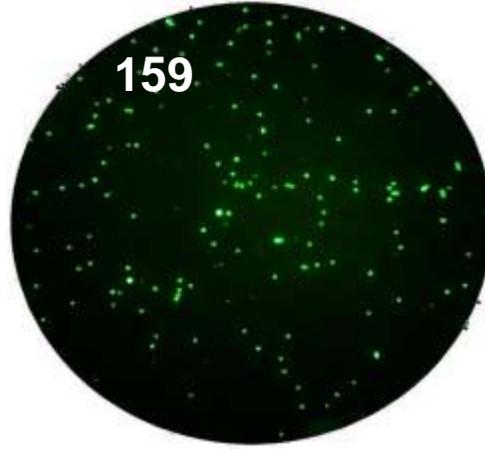
TVA™ Assay: Plate layout



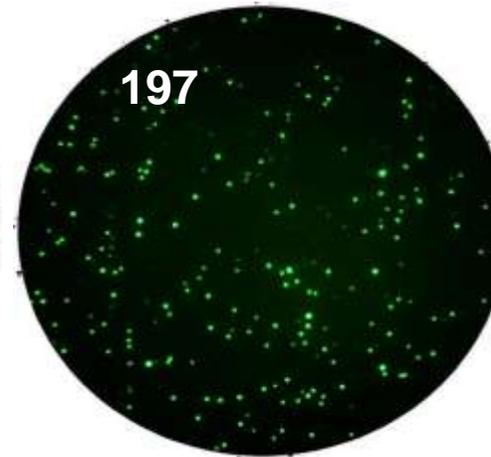
Representative images, 96-well assay



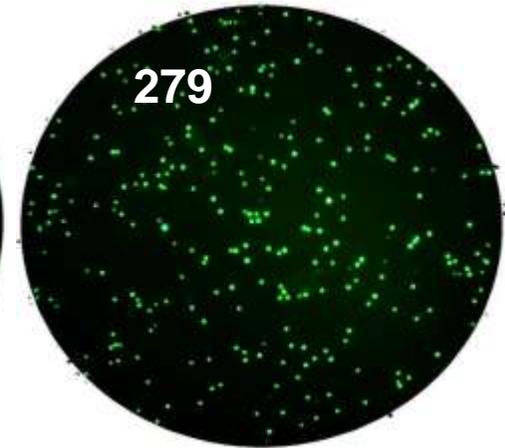
50:1 (65% killing)



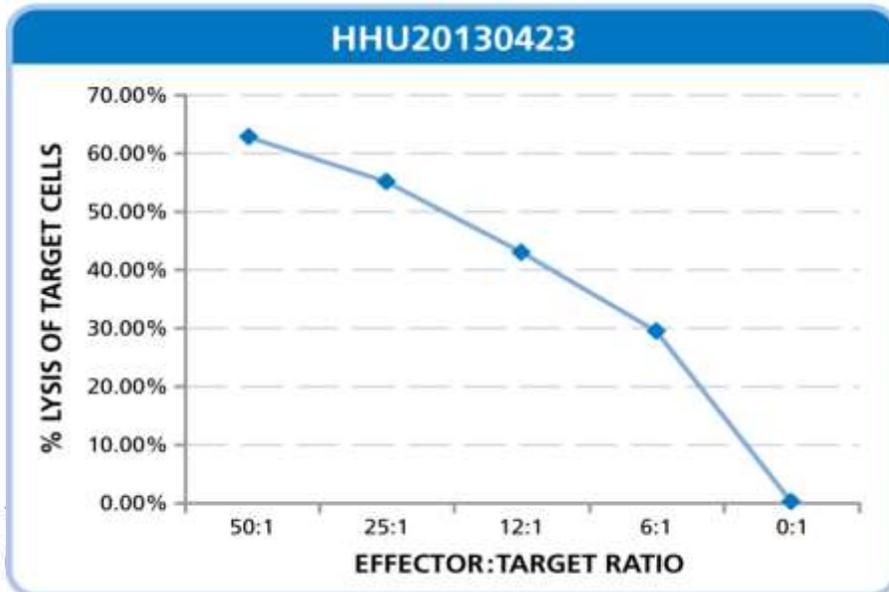
12:1 (43% killing)



6:1 (29% killing)

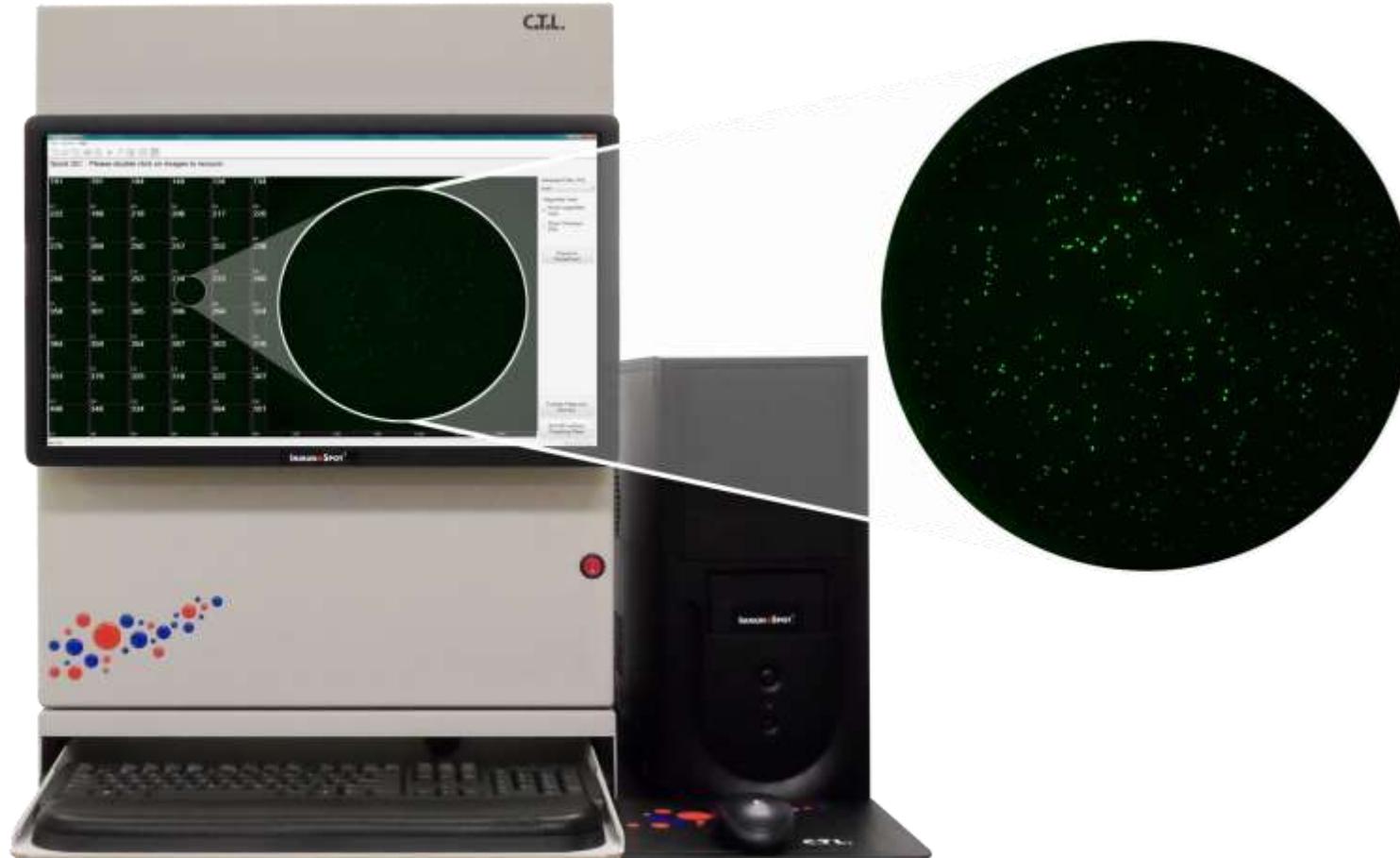


0:1



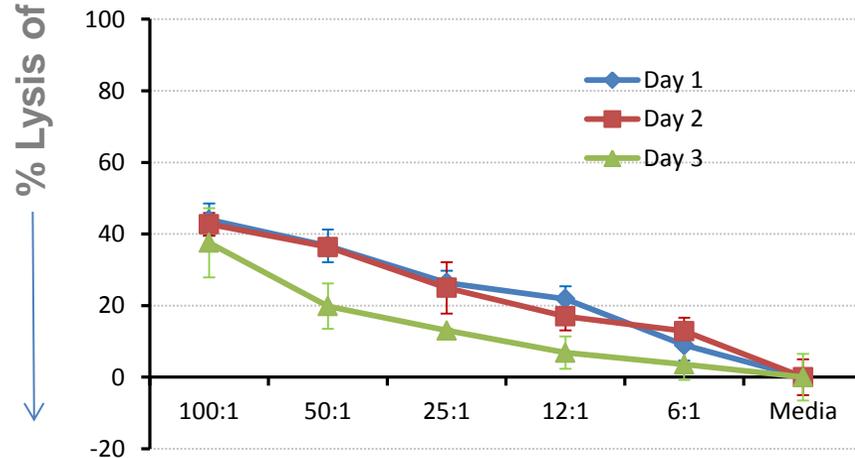
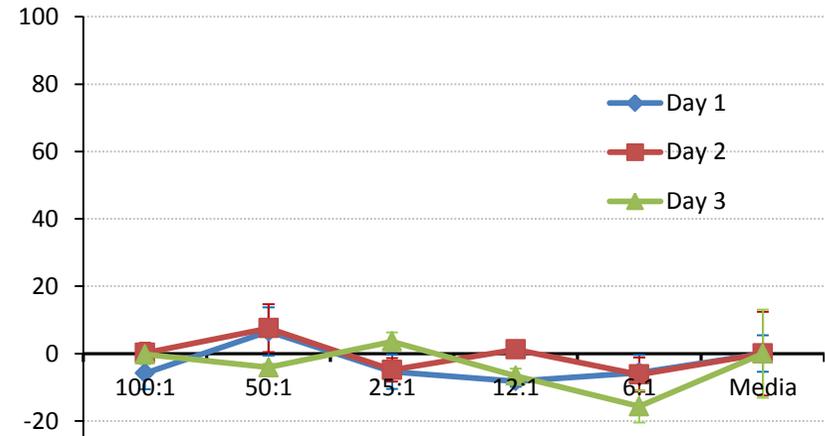
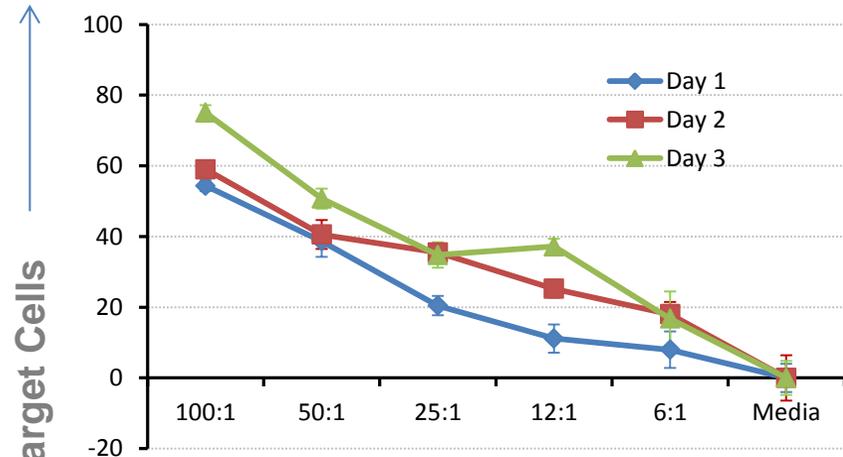
When NK cells are active in PBMC, increased numbers of target cells are lysed as the number of PBMC increase. The dose response curve on the left is automatically generated by the NK-TVA™ instrument.

Single-Cell Imaging



The CTL Analyzer is capable of single-cell imaging in microtiter plates

Precision – Repeatability on multiple days



Cryopreserved PBMC of three human donors from the CTL ePBMC[®] Library were each tested in three repeat experiments. NK-activity characterized PBMC are offered by CTL as positive and negative controls, or for assay development, qualification and validation.

Effector : Target Ratio

Mini TVA™

Terasaki plate-based counting permits 1:10 miniaturization vs. the 96-well TVA™

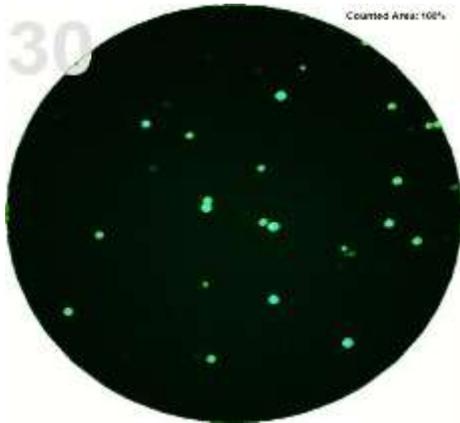
- Assay is carried out in Terasaki plates (20µl/well)
- Counting is performed using ImmunoSpot® TVA™ Software

Mini TVA™ is Ideal when limited numbers of PBMC are available, e.g., pediatric or oncologic samples, HIV, etc.



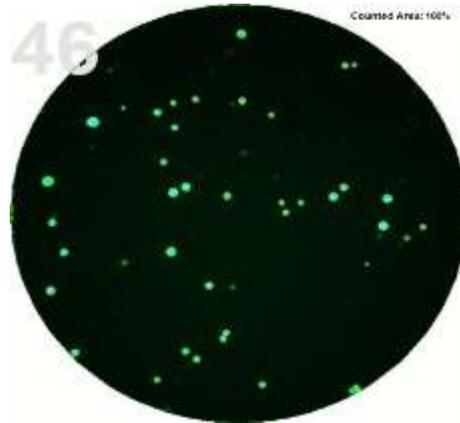
Mini TVA™:

Target cells in Terasaki plates



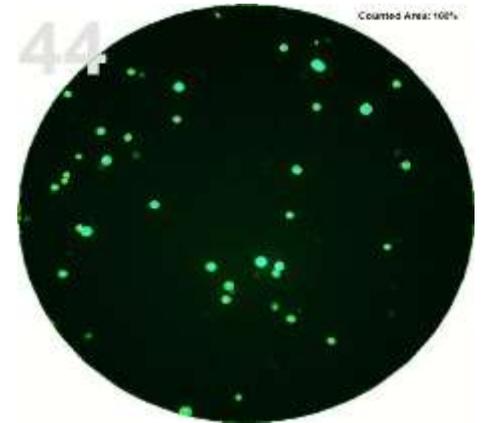
30

Counted Area: 100%



46

Counted Area: 100%



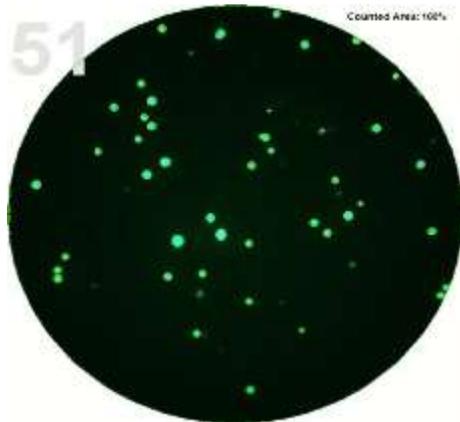
44

Counted Area: 100%

E:T Ratio 90:1 (53% killing)

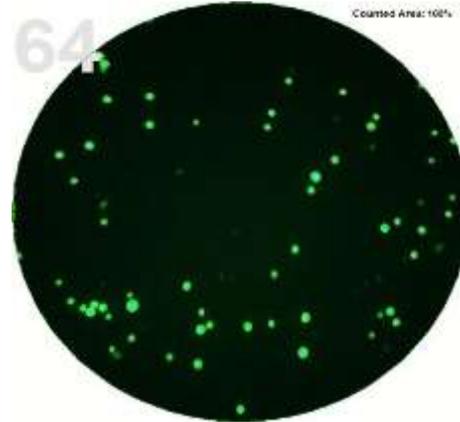
45:1 (28% killing)

22:1 (32% killing)



51

Counted Area: 100%



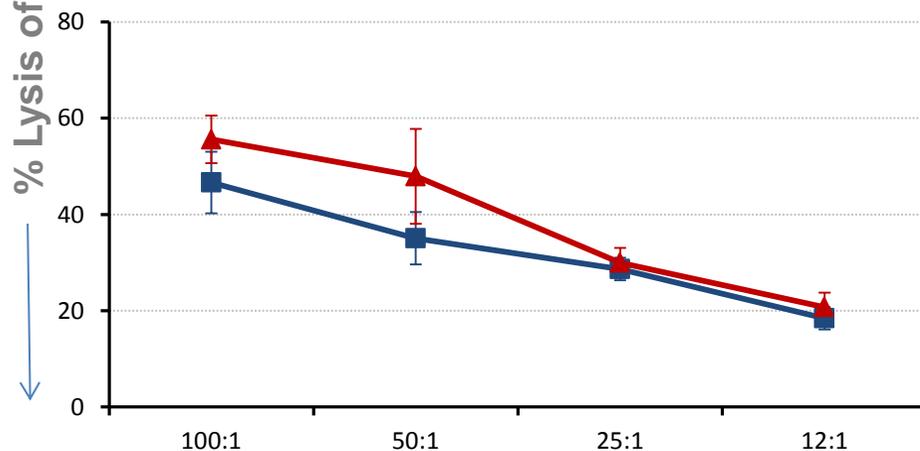
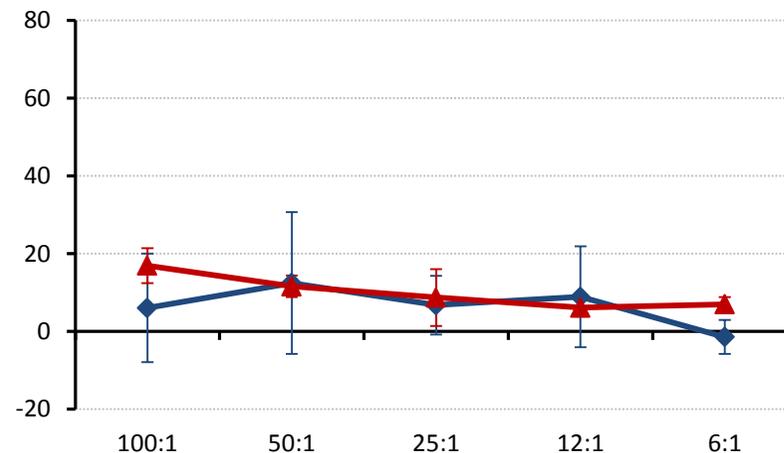
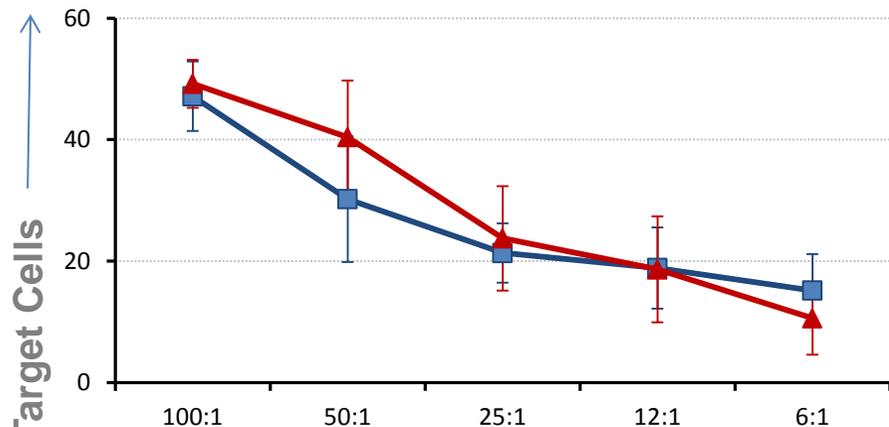
64

Counted Area: 100%

E:T Ratio 11:1 (20% killing)

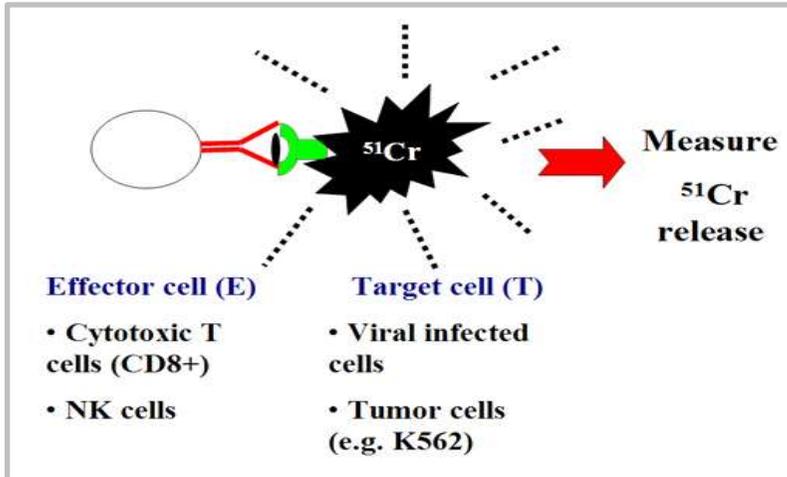
0:1

TVA™ in Terasaki vs. 96-well plates testing three different donors



Effector : Target Ratio

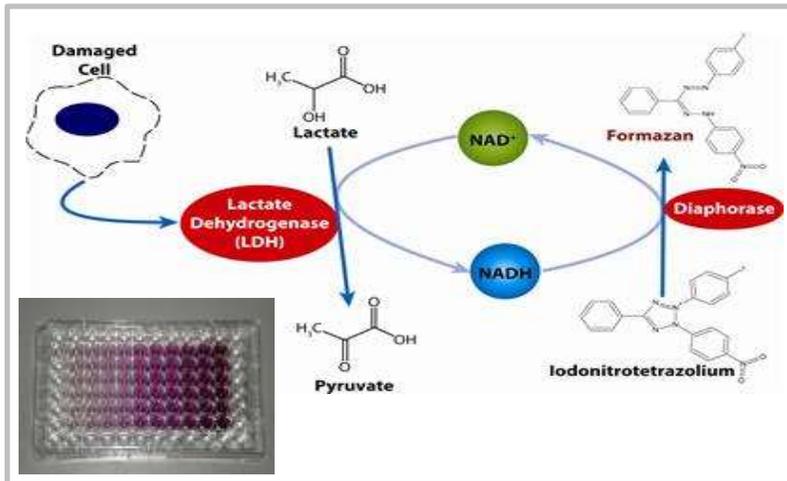
Conventional Approaches (#1)



Chromium Release Assay

- Target cells are loaded with radioactive Chromium
- Measure of released radioactivity is correlated to % killing

- Both are indirect, semi-quantitative, and have low signal-to-noise performance
- Cr release involves radioactive components and waste



Lactate Dehydrogenase Assay

- Target cells, upon lysing, release enzymes: Lactate Dehydrogenase
- Enzymes react with Formazan to elicit a purple color
- Colorimetric measurements of Formazan is correlated to % killing

Conventional Approaches (#2)

Imaging-based vs. flow cytometry-based detection of target cell lysis

- High-throughput, much faster
- Fully-automated data analysis
- GLP, audit trails automatically provided
- Fewer effector cells (much fewer for Mini TVA™)
- Essentially maintenance-free instrument
- More cost-effective

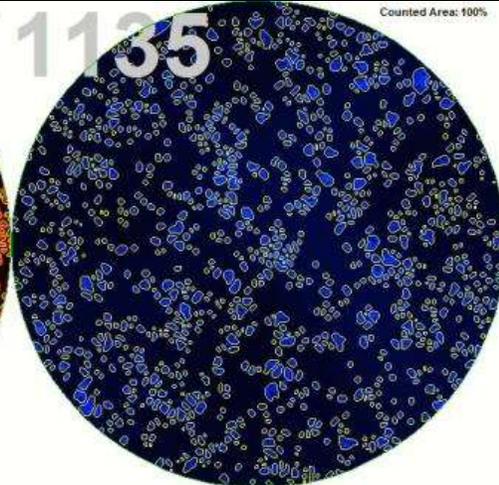
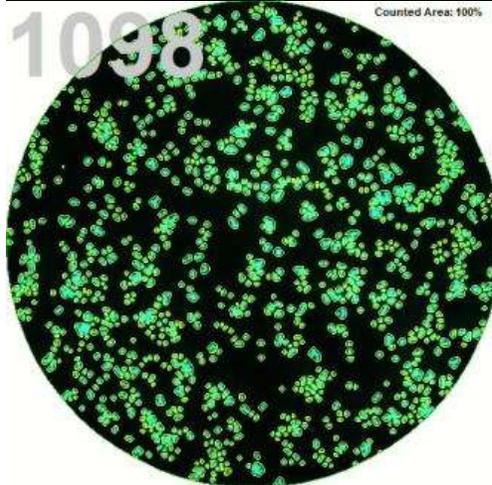
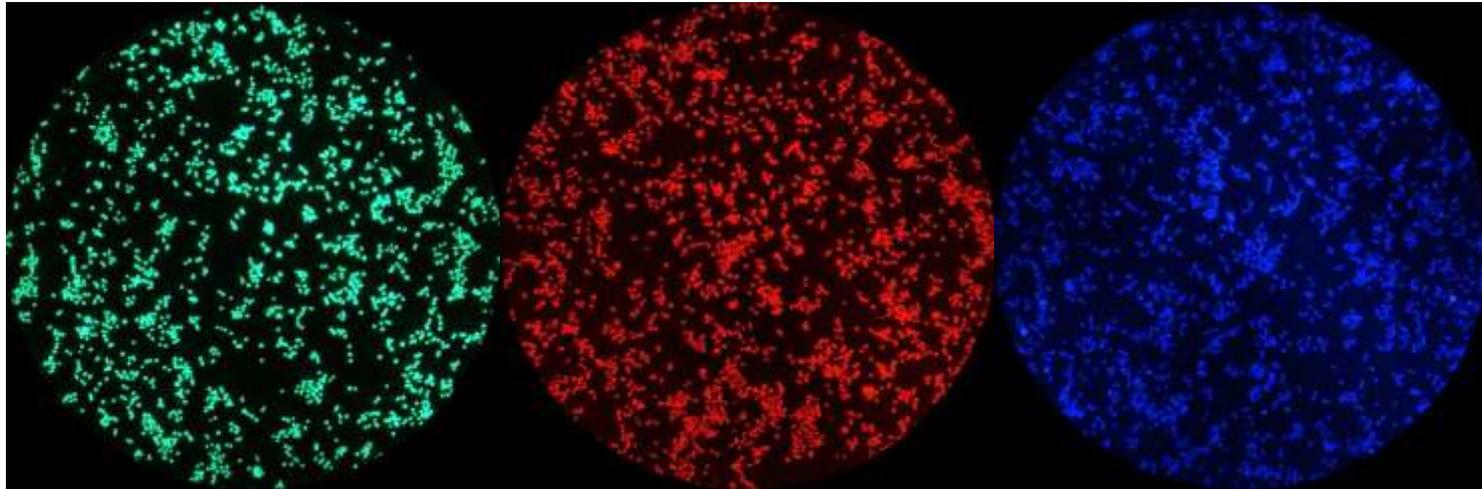
Advantages of TVA™ over Conventional NK Assays

- Non-radioactive
- Fewer effector cells needed
- Direct detection of target cells by imaging resulting in quantitative analysis
- Automated scanning, counting, and analysis

TVA™ vs. Chromium Release



Multicolor TVA™ mode



Calcein green

Calcein Red Orange

Calcein Violet

Contact us today
to learn more about

The NK Target cell Visualization Assay

TVA™

by

CTL. 
Cellular Technology Limited

www.immunospot.com