

Agilent xCELLigence Immunotherapy Kit

Monitor liquid tumor cell killing in real time





Monitor the efficacy of liquid cancer immunotherapies inside your incubator

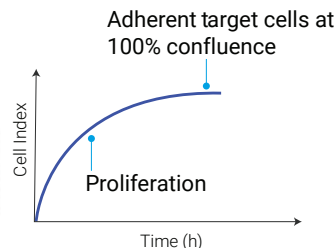
Pair Agilent xCELLigence immunotherapy kits with your real-time cell analysis (RTCA) system for a noninvasive approach to understanding immune cell-mediated cytotoxicity against hematopoietic tumors. Enhance assay reproducibility in your liquid cancer immunotherapy and suspension tumor-killing applications, while preserving cell functionality under physiological conditions through continuous kinetic monitoring, without the interference of labeling or staining.

- **Real time:** Quantitative monitoring of both fast (hours) and slow (days) killing
- **Label-free:** Avoid interference of labeling or staining
- **Simple workflow:** Reduce the number of sample handling steps
- **Improved sensitivity:** Physiologically relevant, low effector-to-target ratios
- **Automatic data plotting:** Eliminate subjective data

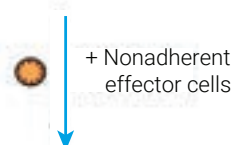
Liquid tumor cytotoxicity assay using the Agilent xCELLigence RTCA and tethering kit

Step 1

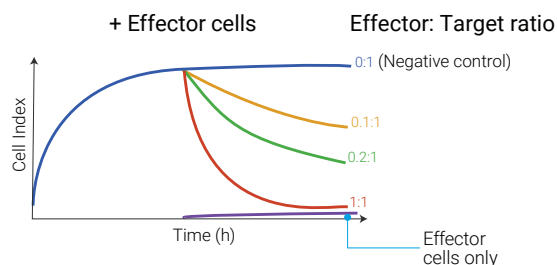
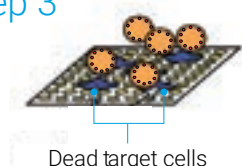
Target liquid cancer cells
(immobilized by antibody)



Step 2



Step 3



Step 1

The target liquid cancer cells are first seeded in the wells of an electronic microplate (E-Plate) that have been precoated with a tethering antibody. This biosensor signal, also known as Cell Index, increases as cells proliferate and then plateaus as cells approach 100% confluence.

Step 2

When added, nonadherent immune effector cells in suspension do not cause impedance changes (due to lack of adherence to the gold biosensors).

Step 3

If effector cells induce cell death of the target tumor cells, this cytolytic activity is precisely detected with high sensitivity. The continuous acquisition of impedance data for each well enables the generation of real-time killing curves for multiple conditions simultaneously.

Immune effector cells and liquid cancer target cells in use

Cancer Type	Validated Target Cell Line	Liquid Tumor Tethering Specificity
Acute Lymphoblastic Leukemia (ALL)	NALM6	CD9
Chronic Myelogenous Leukemia (CML)	K562	CD71
Non-Hodgkin Lymphoma (NHL)	Raji	CD19
Multiple Myeloma (MM)	RPMI 8226	CD9

Liquid tumor killing assay application data

In this example, the wells of an Agilent E-Plate are precoated with a tethering reagent, enabling liquid tumor targets to be immobilized on the plate bottom (Figure 1A). Immobilized target cells generate a robust impedance signal and proliferate to the point of confluence, resulting in a plateaued impedance signal. The growth of untethered target cells is essentially undetectable (Figure 1B). With or without tethering reagent coating the wells, the effector cells (NK-92 cells) produce minimal signal on their own (Figure 1B). The addition of effectors to the immobilized targets results in target cell death in a dose-dependent manner (Figure 1C).

Side-by-side, four-hour assays were performed for NK-92 cell mediated killing of Raji B cells that were immobilized (analyzed by Agilent xCELLigence) or in suspension (analyzed by flow cytometry). This assay was used to assess whether the physical immobilization of liquid tumor cells, via tethering reagent, affects the efficiency with which they are killed. As shown in Figure 1D, the killing trends observed by these two methods correlate closely. The results were consistent with several previous publications, demonstrating that xCELLigence data reinforces data obtained by traditional assays.

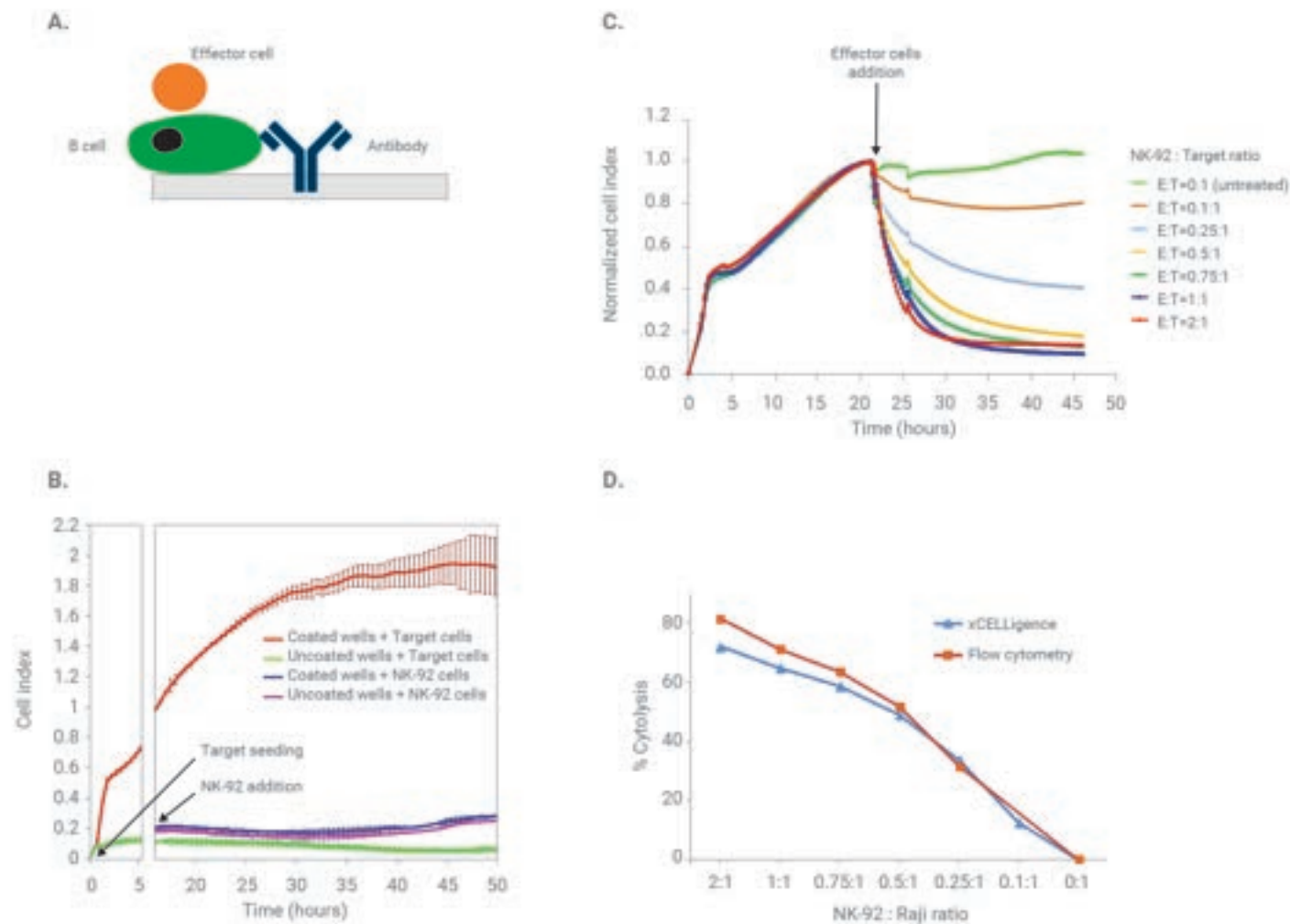


Figure 1. NK cell-mediated cytotoxicity of B cell lymphoma using the Agilent tethering kit.



Agilent xCELLigence instruments for immunotherapy assays

				
Dual Purpose (DP)	Single Plate (SP)	Multiple Plates (MP)	High Throughput (HT)*	Impedance and Imaging (eSight)
3 × 16 wells	1 × 96 wells	6 × 96 wells	Up to 4 × 384 wells	Up to 5 × 96 wells

*Agilent xCELLigence RTCA HT-BioTek BioSpa 8 monitors up to 8 × 384 wells



Liquid tumor killing assay (anti-CD40)

Tethering Kit (Up to 6 × 96-Well Plates) Part Number 8100005
125x Tethering reagent (anti-CD40) (250 µL)
10x Tethering buffer (10 mL)
Cytolysis reagent (1.5 mL)

Sample Kit (Up to 2 × 96-Well Plates) Part Number 8100006
125x Tethering reagent (anti-CD40) (90 µL)
10x Tethering buffer (10 mL)
Cytolysis reagent (1.5 mL)

Liquid tumor killing assay (anti-CD19)

Tethering Kit (Up to 6 × 96-Well Plates) Part Number 8100011
125x Tethering reagent (anti-CD19) (250 µL)
10x Tethering buffer (10 mL)
Cytolysis reagent (1.5 mL)

Sample Kit (Up to 2 × 96-Well Plates) Part Number 8100012
125x Tethering reagent (anti-CD19) (90 µL)
10x Tethering buffer (10 mL)
Cytolysis reagent (1.5 mL)

Liquid tumor killing assay (anti-CD9)

Tethering Kit (Up to 6 × 96-Well Plates) Part Number 8100014	Sample Kit (Up to 6 × 96-Well Plates) Part Number 8100015
125x Tethering reagent (anti-CD9) (250 µL)	125x Tethering reagent (anti-CD9) (90 µL)
10x Tethering buffer (10 mL)	10x Tethering buffer (10 mL)
Cytolysis reagent (1.5 mL)	Cytolysis reagent (1.5 mL)

Liquid tumor killing assay (anti-CD71)

Tethering Kit (Up to 6 × 96-Well Plates) Part Number 8100017	Sample Kit (Up to 6 × 96-Well Plates) Part Number 8100018
125x Tethering reagent (anti-CD71) (250 µL)	125x Tethering reagent (anti-CD71) (90 µL)
10x Tethering buffer (10 mL)	10x Tethering buffer (10 mL)
Cytolysis reagent (1.5 mL)	Cytolysis reagent (1.5 mL)

Related products

Part Number	Part
300600890	E-Plate 16 PET (6 plates)
300600880	E-Plate 16 PET (36 plates)
5469830001	E-Plate 16 (6 plates)
5469813001	E-Plate 16 (36 plates)
300601140	E-Plate VIEW 16 (6 plates)
300601150	E-Plate VIEW 16 (36 plates)
300600910	E-Plate 96 PET (6 plates)
300600900	E-Plate 96 PET (36 plates)
5232368001	E-Plate 96 (6 plates)
5232376001	E-Plate 96 (36 plates)
300601020	E-Plate VIEW 96 (6 plates)
300601030	E-Plate VIEW 96 (36 plates)
5867681001	E-Plate 384 (10 plates)
5867673001	E-Plate 384 (40 plates)

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