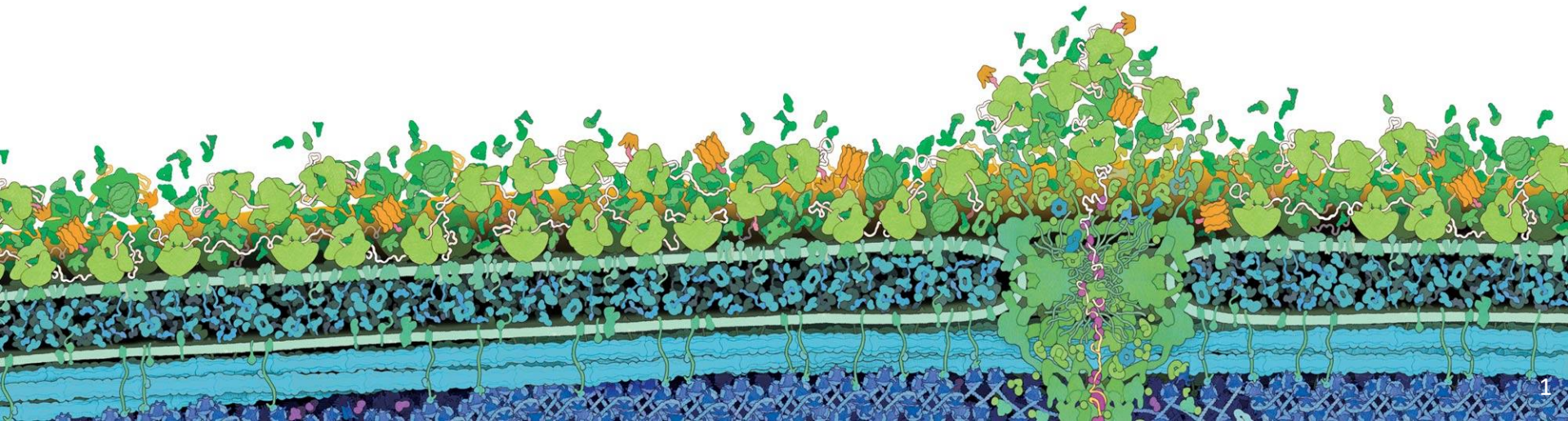
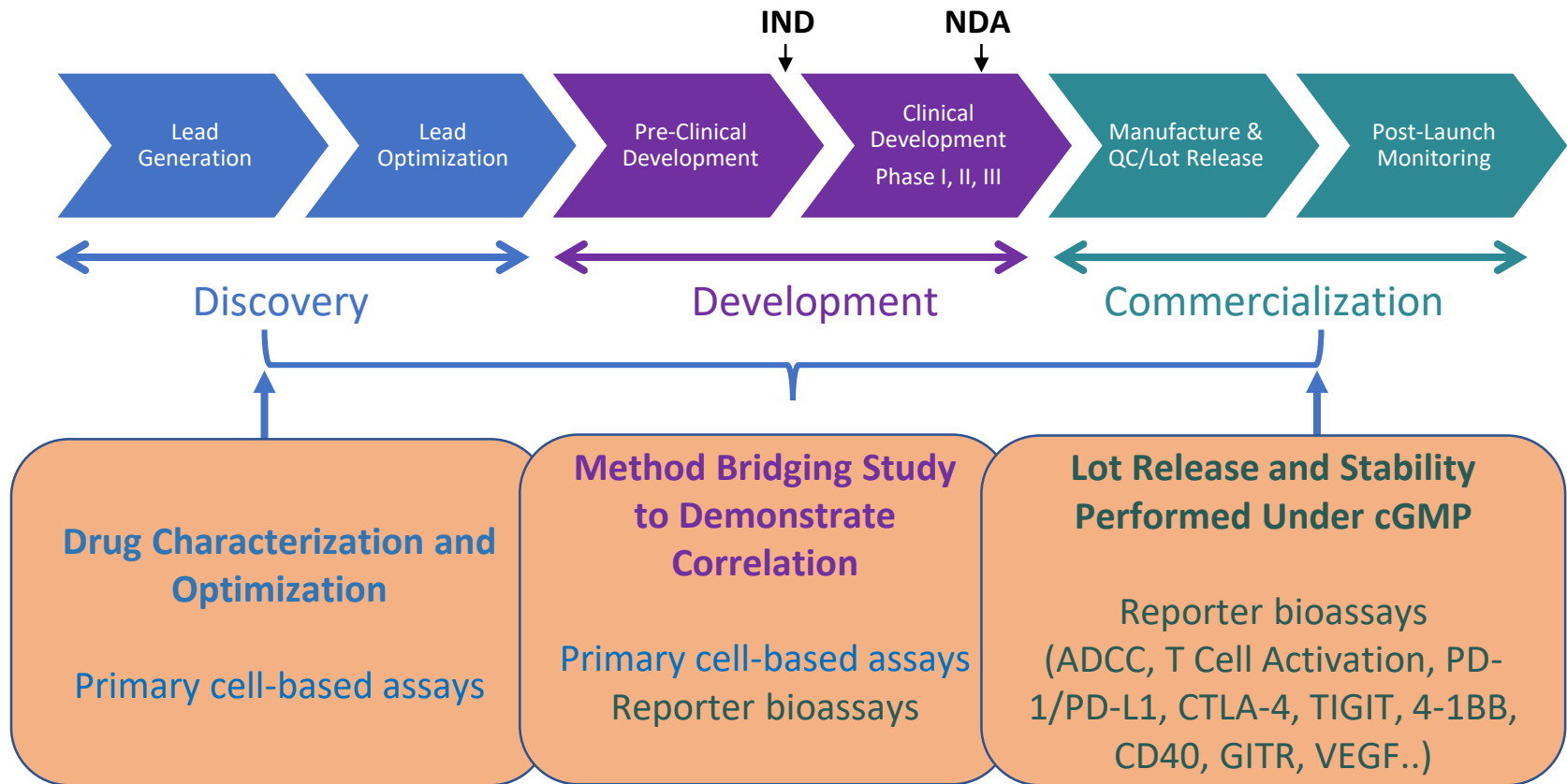


Bioluminescent Bioassays: From Reporter Cell Lines to Primary Cells

Jey Cheng, Ph.D

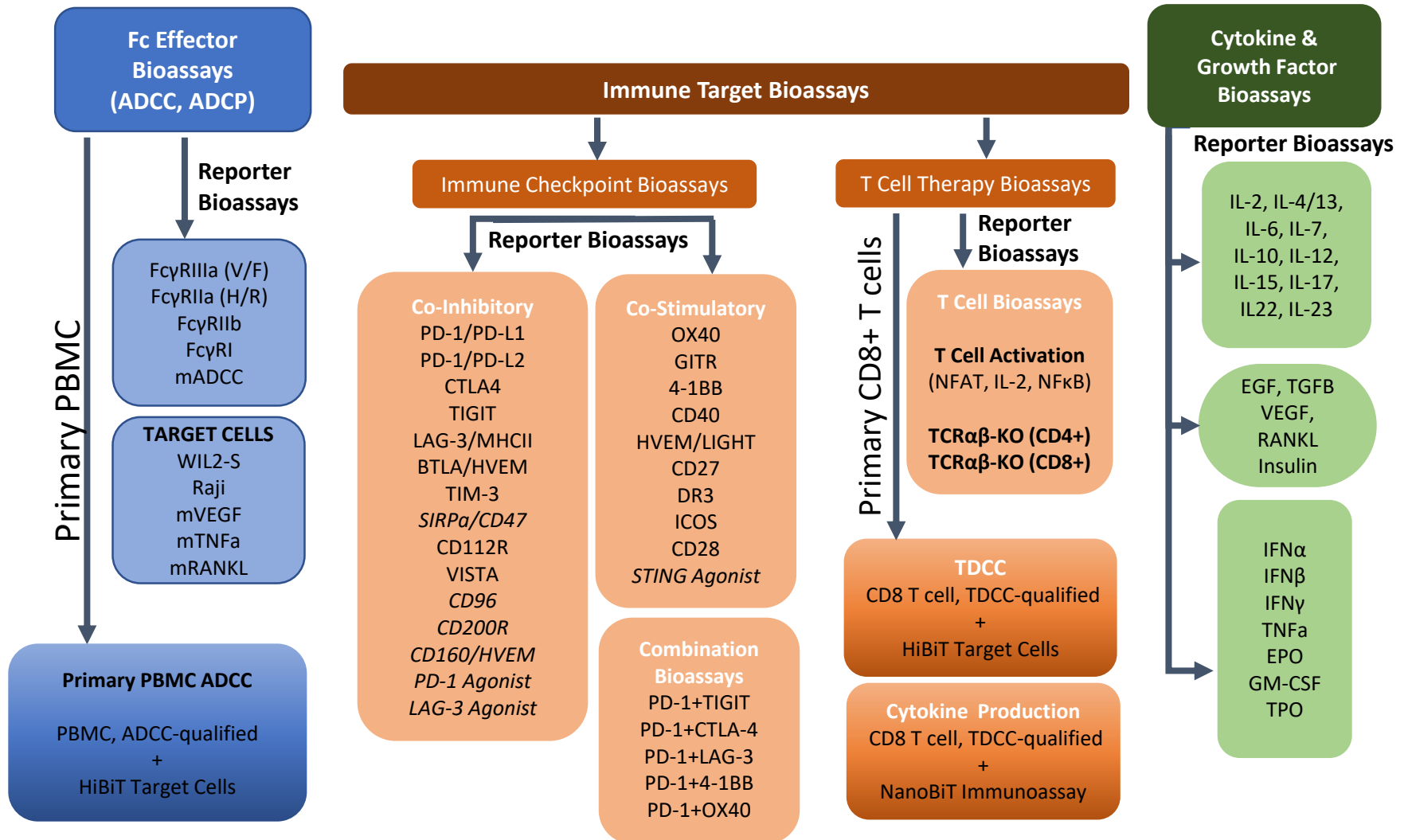


Cell-based Functional Assays Used during Product Life Cycle for Biologic Immunotherapy

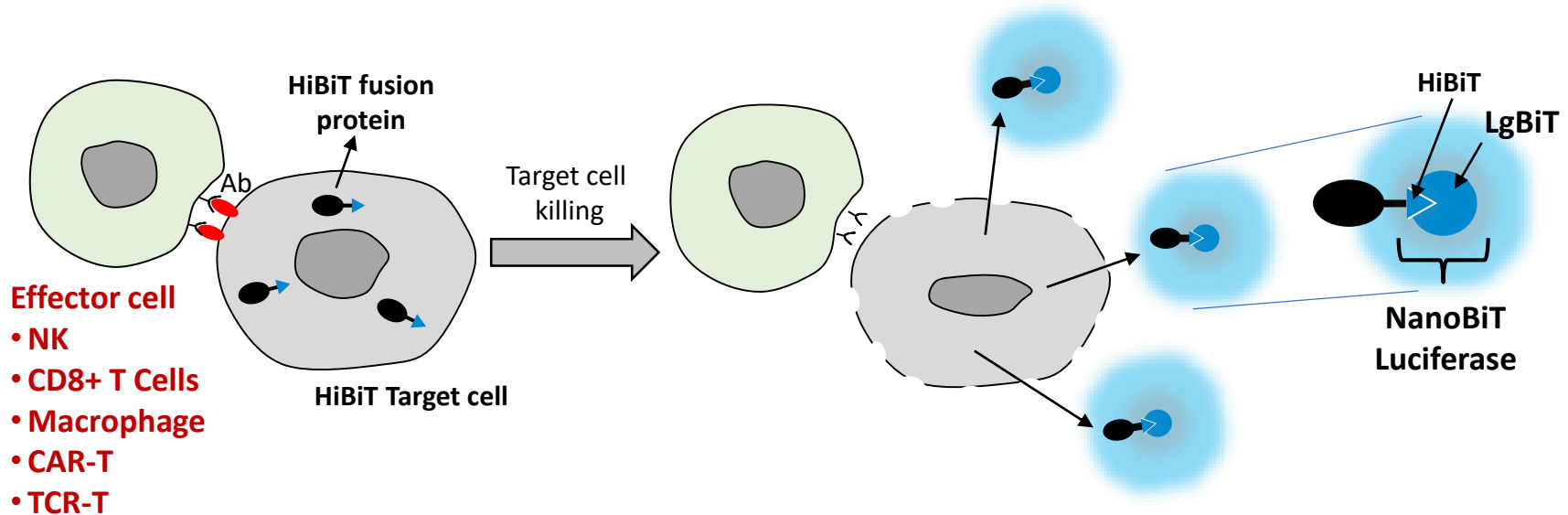


- Many reporter bioassays have been developed and used as potency bioassays for lot release and stability study.
- There are still challenges to develop primary cell-based assays due to the variation from donors and poor assay sensitivities.

Expand Reporter Bioassays to Primary Cells-based Assays



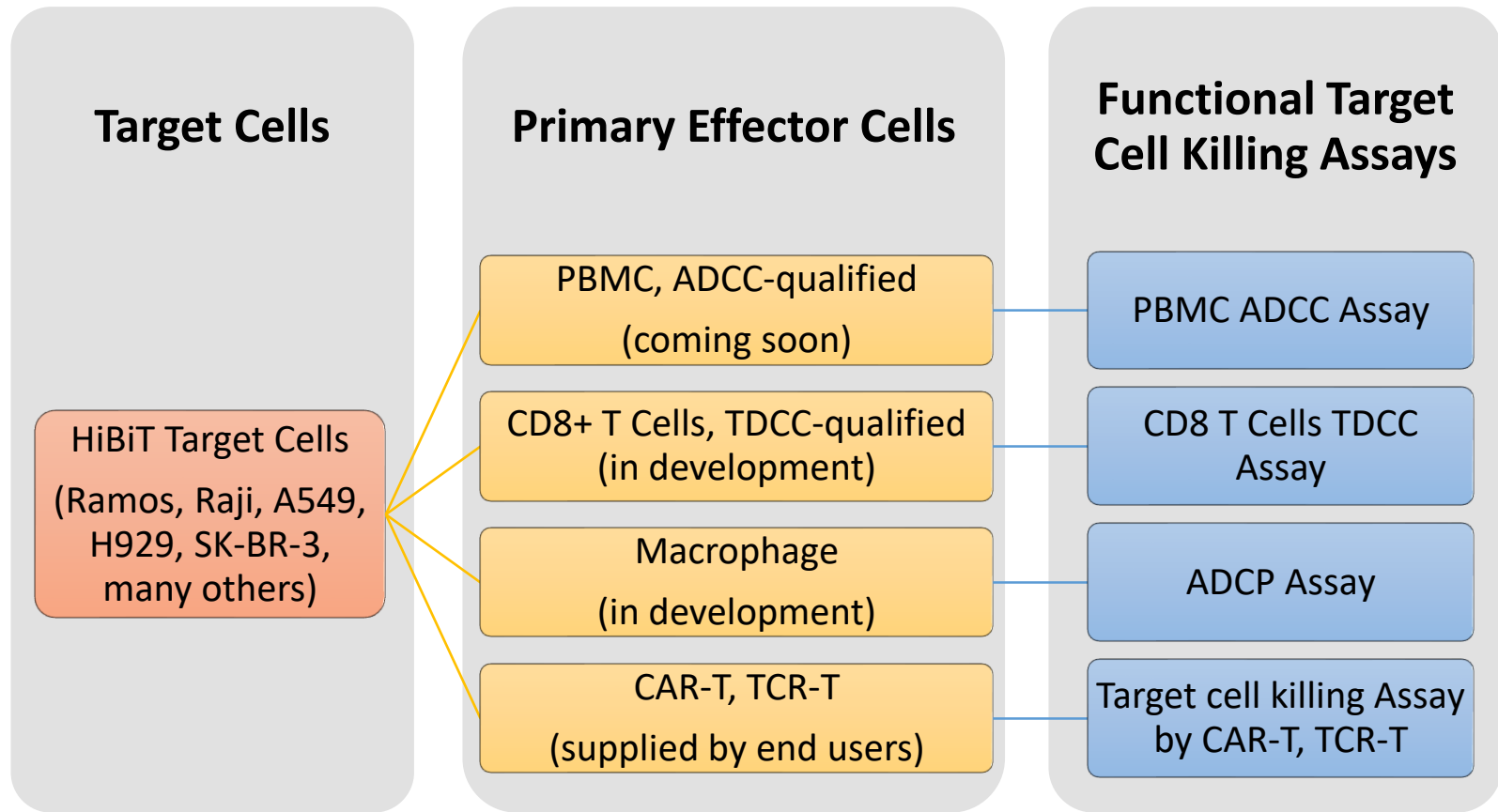
HiBiT-based Target Cell Killing (TCK) Assay Principle



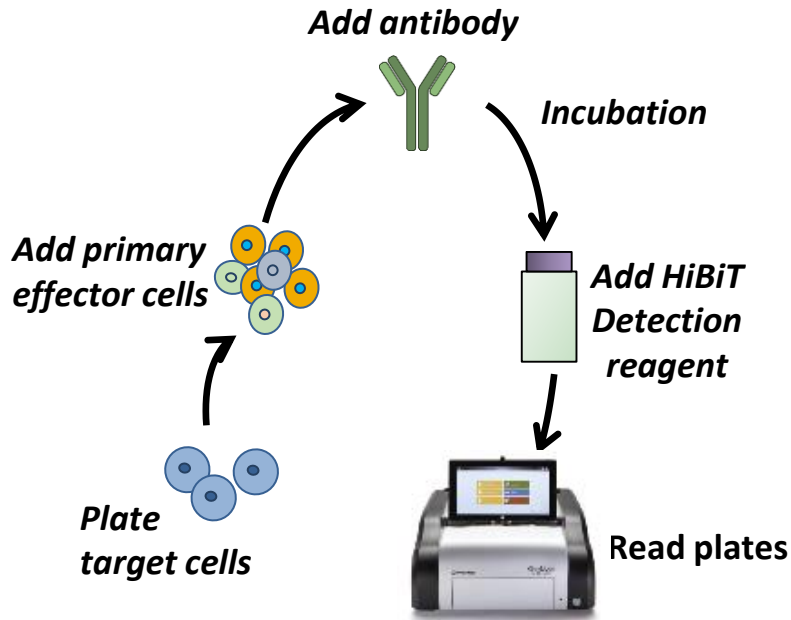
- The lysis of HiBiT target cells releases HiBiT fusion protein into the medium, which binds to cell-impermeable LgBiT in the detection reagent and forms functional NanoBiT™ luciferase and emits lights.
- Simple, homogenous, no medium transfer required



HiBiT Target Cells can Work with Different Primary Effector Cells in Target Cell Killing Assays



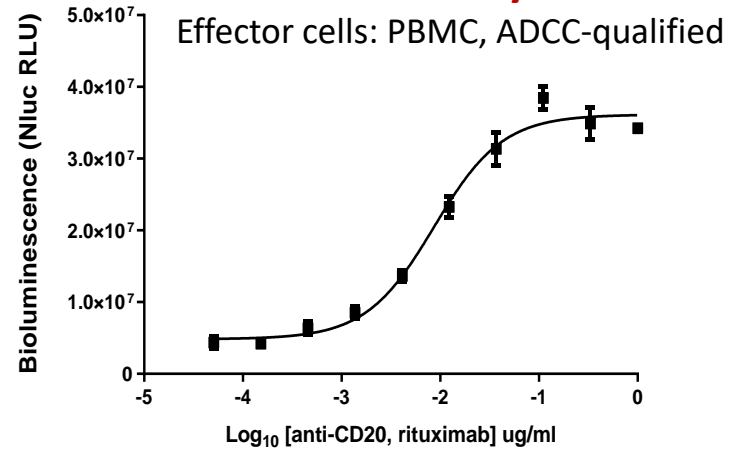
Using HiBiT Target Cells in ADCC and TDCC Assays



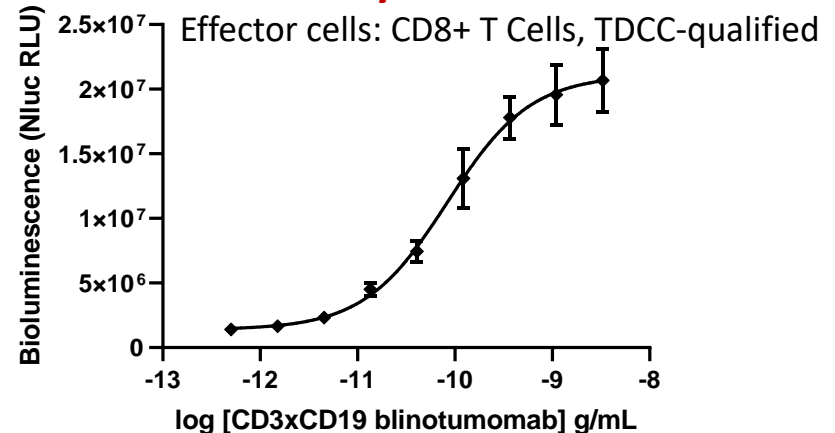
Features

- Homogenous assay
- target cell-specific killing
- Low spontaneous release (<10% MR)
- Simple, and fast

PBMC ADCC assay



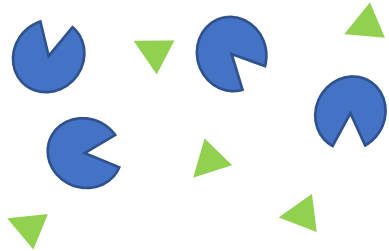
TDCC assay





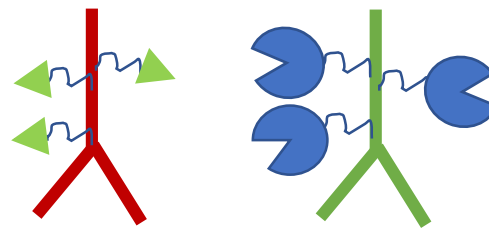
NanoBiT Immunoassays for Measuring Cytokine Release

Recombinant NanoBiT[®] luciferase subunits (SmBiT and LgBiT)



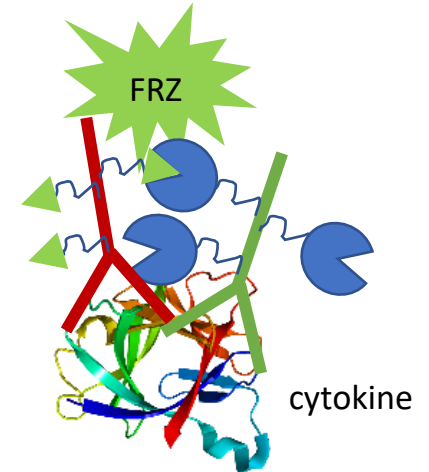
- Extremely low-affinity interaction
- Negligible association in solution

NanoBiT[®]-labeled primary antibodies (mAb1-SmBiT and mAb2-LgBiT)



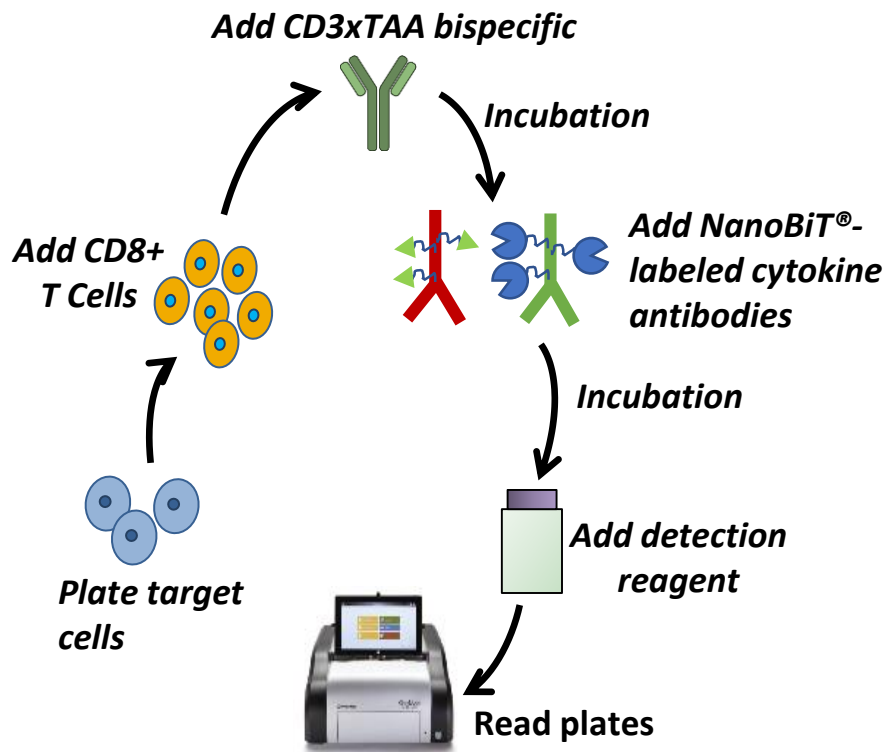
- Extremely low-affinity interaction
- Negligible association in solution
- Very low assay background

Analyte facilitated colocalization of mAb1-SmBiT and mAb2-LgBiT
→ NanoBiT[®] luciferase reconstitution



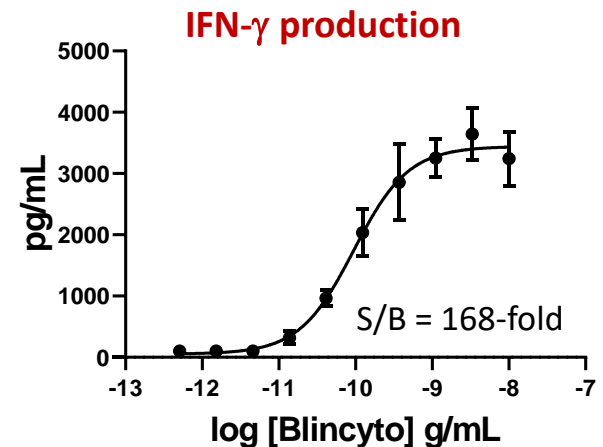
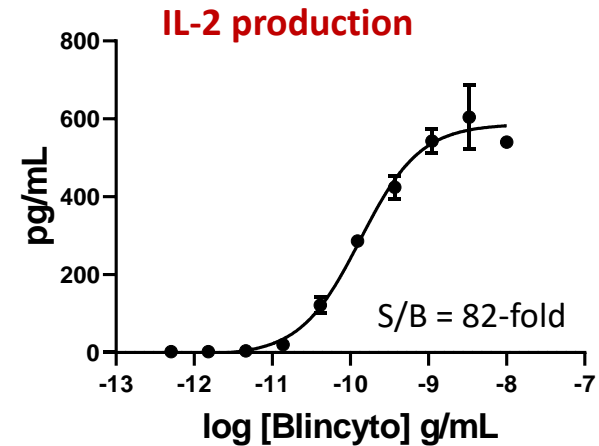
- Luminescent signal proportional to analyte levels
- Homogeneous assay format

Measure IFN- γ and IL-2 Production from CD8+ T Cells Activated by Bispecific Antibody



Features

- Homogenous
- Simple, fast and sensitive





Summary

Newly developed primary cell-based assays offer

- High-quality primary effectors cells
 - produced with controlled manufacturing procedures
 - QC tested in functional assays
 - Developed in Thaw-and-Use format without the need of cell culture
- Convenient and sensitive assay read-out
 - HiBiT-based target cell killing assays use HiBiT complementation technology and can measure target cell-specific killing in mixed culture with different effector cells.
 - NanoBiT Immunoassay shows linear correlation between assay signal and cytokine levels and can be used for homogenous detection of cytokine production.