Development of a Robust Reporter-based T cell Activation Assay for Therapeutic Biologics in Immunotherapy

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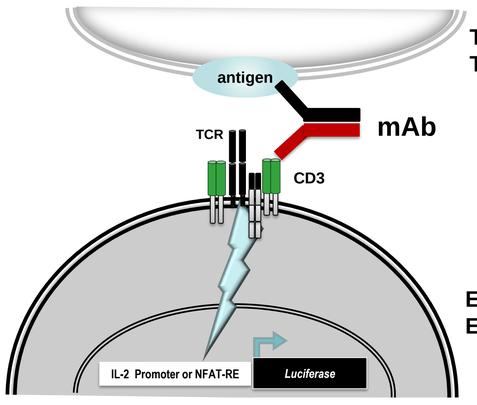
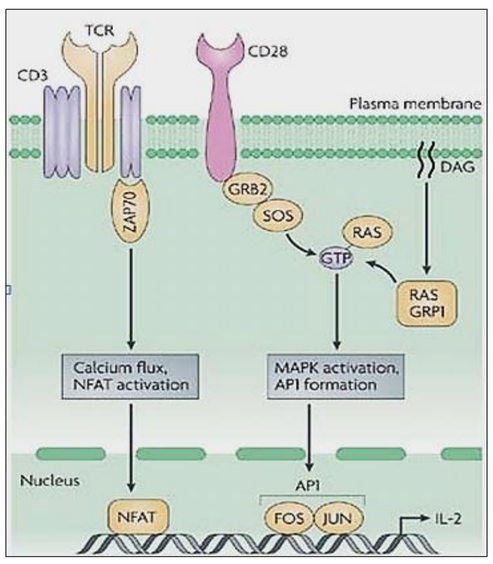
1. Assay Principle



- Jurkat T-cells stably expressing luciferase reporter driven by IL2 promoter or NFAT-RE, are used as effector cells.
- Tumor cell lines endogenously expressing cancer antigen are used as antigen presenting cells (APC).
- By co-cultivating the two cell lines in the presence of CD3 bispecific antibody, TCR/CD3 is activated in Jurkat effector cells.
- Luciferase activity is up regulated through IL-2 promoter or NFAT-RE activation.

2. Genetically Engineered Jurkat T Cell Reporter Cell lines as Effector Cells



Lineberry-N *et al. Nature Reviews Immunology 2007*

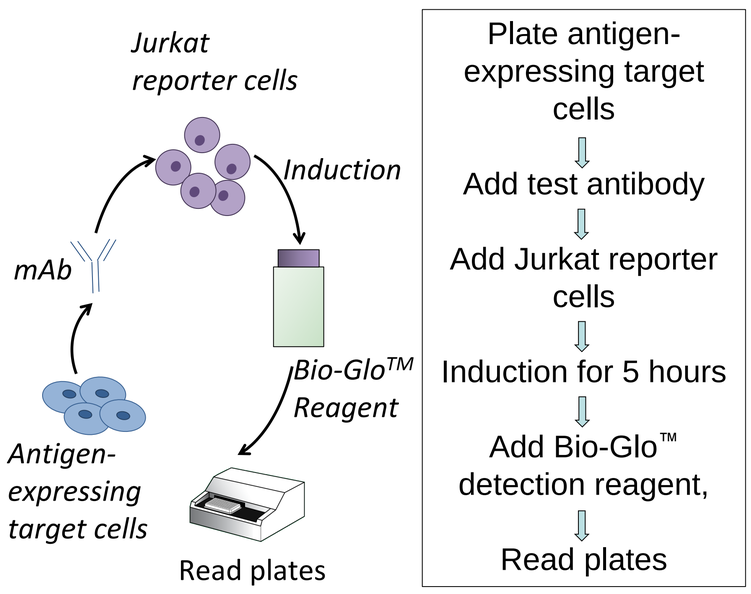
1. Jurkat / IL-2 Reporter Cell Line: Responds to TCR/CD3 activation plus CD28 co-stimulation.

IL-2 promoter — *luc2P*

2. Jurkat / NFAT-RE Reporter Cell Line: Responds to TCR/CD3 activation.

NFAT-RE — *luc2*

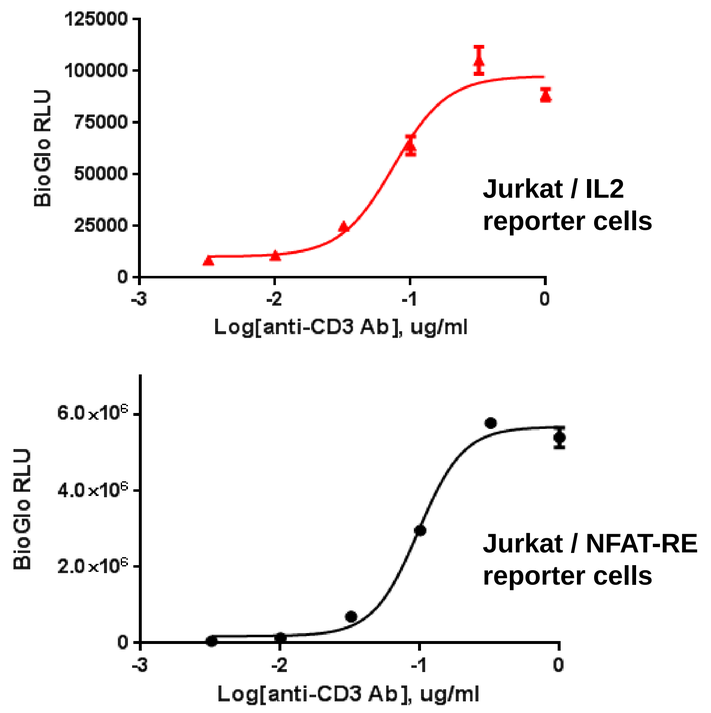
3. Assay Format and Features



Assay Features:

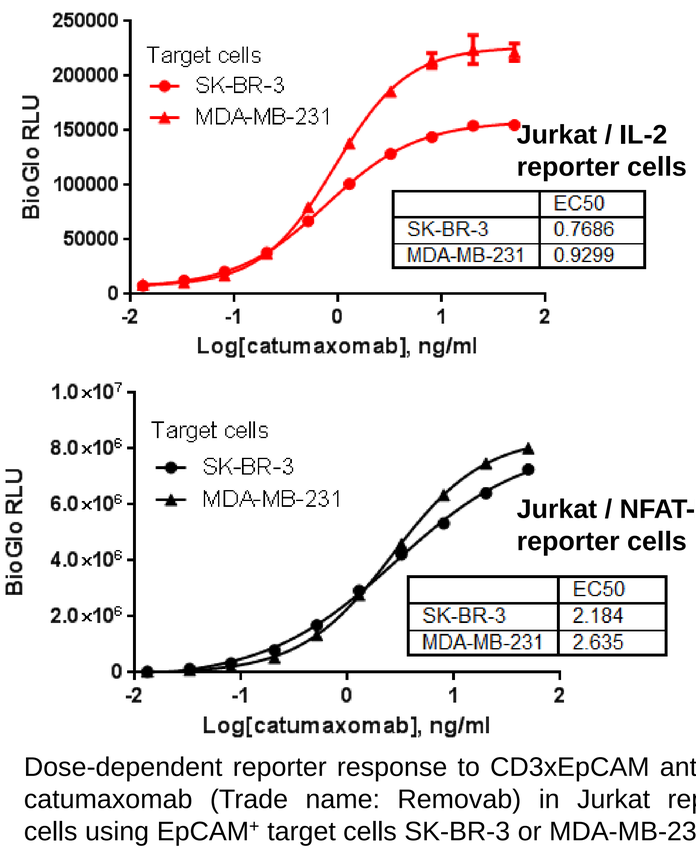
- No need for primary effector cells
- Simple, homogenous
- Specific and robust assay signal
- Short assay time, finish in one day

4. Robust Reporter Response upon Stimulation with CD3 Antibody



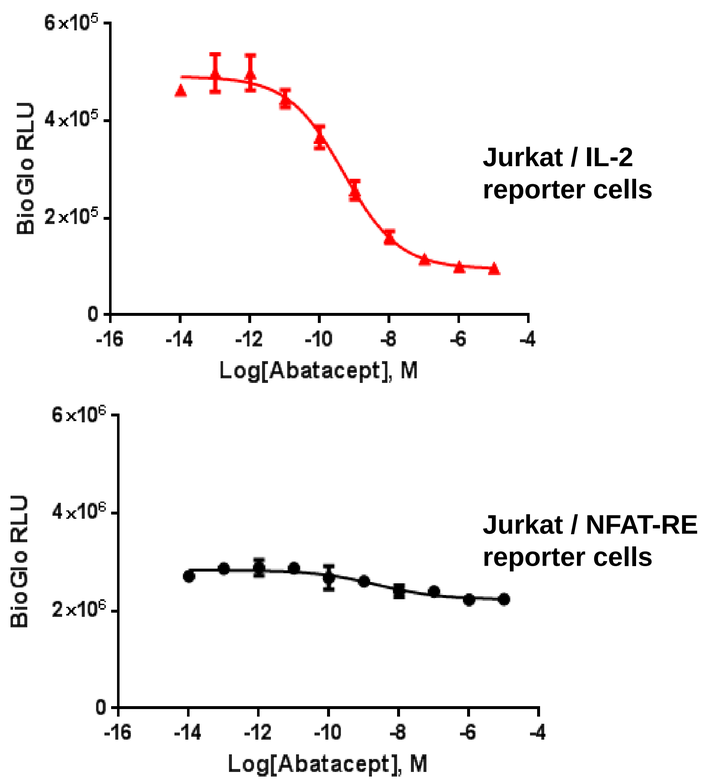
Jurkat reporter cells were incubated with crosslinked anti-CD3 antibody for 5 hours. Luciferase activities were assayed using Bio-Glo™ reagent.

5. Robust Reporter Response to CD3 x EpCAM Ab Catumaxomab using EpCAM+ Target Cells



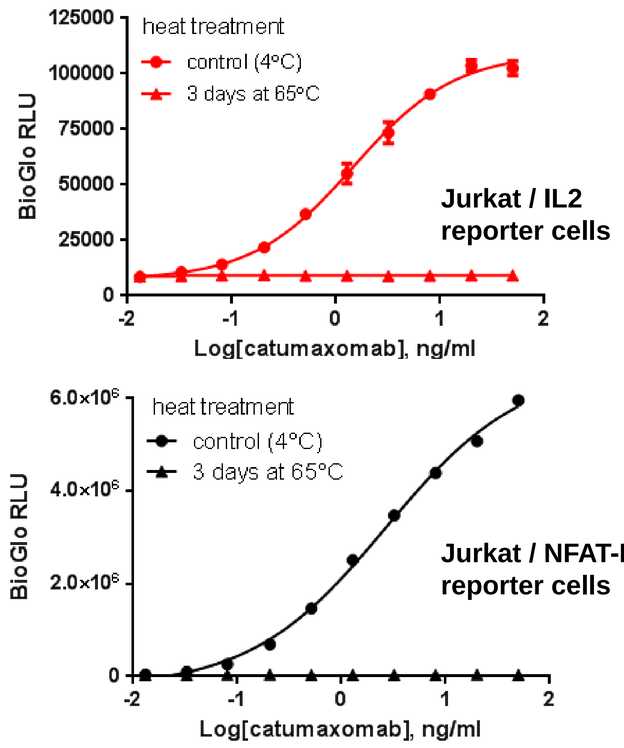
Dose-dependent reporter response to CD3xEpCAM antibody catumaxomab (Trade name: Removab) in Jurkat reporter cells using EpCAM+ target cells SK-BR-3 or MDA-MB-231.

6. Abatacept Inhibition of T cell Co-stimulation can be Detected using IL-2 Reporter, But Not with NFAT-RE Reporter Expressing Effector Cells



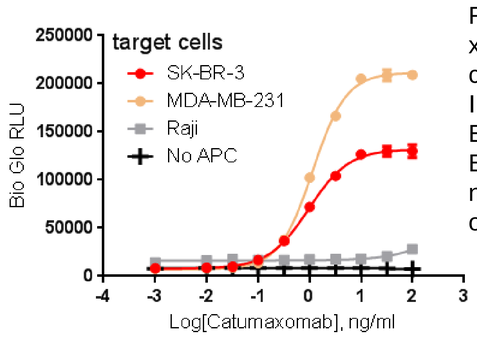
Dose-dependent inhibition of reporter response by abatacept (Trade name: Orencia), a CTLA4-Fc fusion, in Jurkat/IL-2 reporter cells after stimulation with crosslinked CD3 antibody and Raji B cells which express CD28/CTLA4 ligand, B7.

7. Suitability for antibody stability study

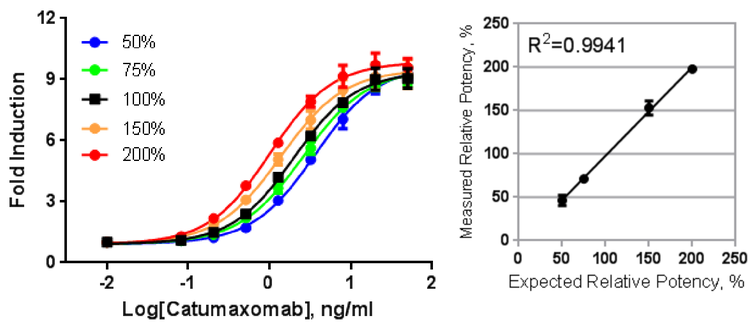


Detecting the loss of reporter response to CD3xEpCAM antibody catumaxomab after heat-treatment in Jurkat reporter cells using EpCAM+ target cells SK-BR-3.

8. Assay Specificity, Ability to Determine Relative Potency and Assay Linearity



Reporter response to CD3 x EpCAM antibody catumaxomab in Jurkat / IL-2 reporter cells using EpCAM+ target cells SK-BR-3 or MDA-MB-231, but not with EpCAM- Raji cells or without APC.



Measuring relative potency for catumaxomab and showing assay linearity using Jurkat / IL-2 reporter cells. Tests are ongoing with Jurkat / NFAT-RE reporter cells.

9. Summary

- **We developed a cell-based T cell activation bioassay using engineered Jurkat reporter cells as effector cells.**
- **Both Jurkat /IL-2 reporter cells and Jurkat /NFAT-RE reporter cells showed robust reporter response to crosslinked CD3 antibody or CD3xEpCAM antibody catumaxomab coated EpCAM+ target cells.**
- **Jurkat /IL-2 reporter cells, but not Jurkat /NFAT-RE reporter cells showed dose-dependent inhibition of CD28 mediated reporter response by CTLA4-Fc fusion abatacept.**
- **Both Jurkat reporter cells are able to detect the loss of biological activity for heat-treated catumaxomab, demonstrating assay suitability in stability study.**
- **The assay is specific, can be used for relative potency determination and shows good assay linearity.**