

## Visualization and quantification of phagocytosis using live-cell analysis and advanced flow cytometry

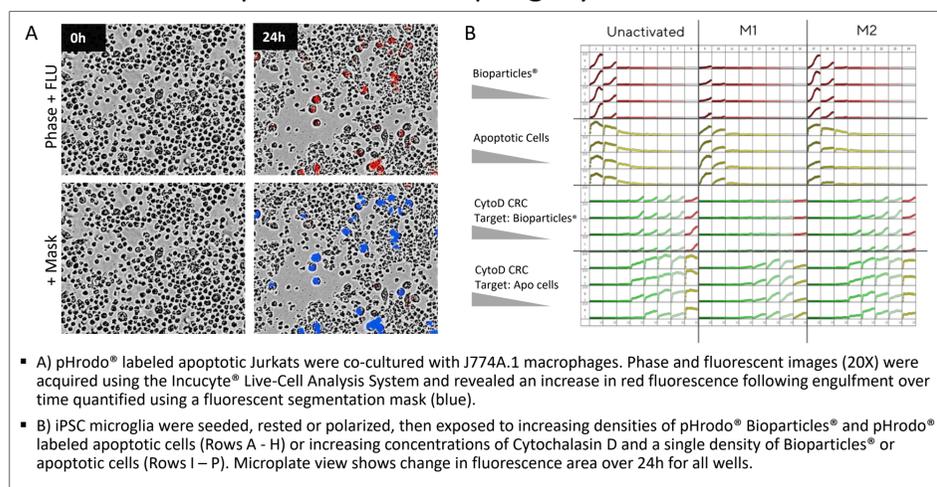
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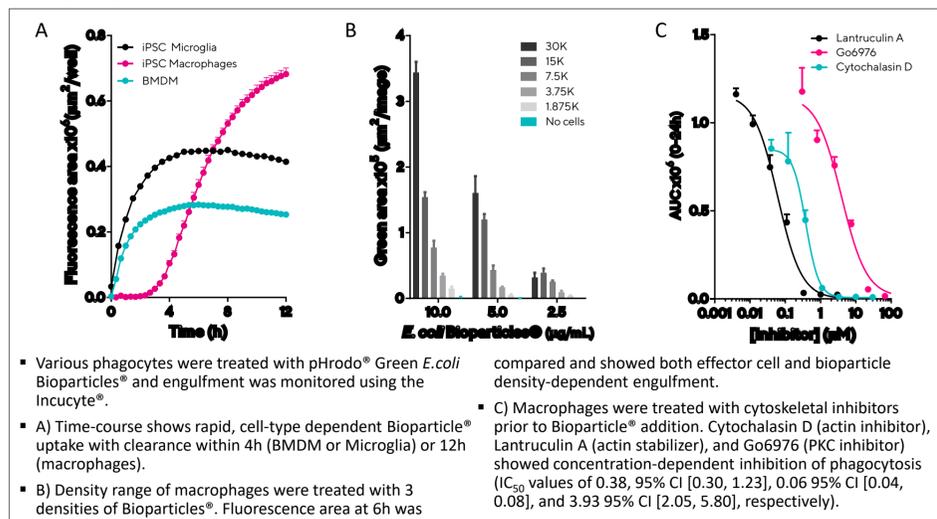
### Summary & Impact

- Phagocytosis, a specific form of endocytosis, is a critical component of innate and adaptive immune responses.
  - Viable cells can be targeted with pro-phagocytic agents, such as monoclonal antibodies (mAbs), that promote engulfment and clearance through antibody-dependent cellular phagocytosis (ADCP), or by blockage of "don't-eat-me" signals (e.g., CD47). Pro-phagocytic mAbs hold great promise as therapeutics.
  - Here, we have developed and validated *in vitro* assays for quantification of phagocytosis in 96-/384-well plates using the Incucyte® Live-Cell Analysis System and/or iQue® Advanced Flow Cytometer.
- Advanced Flow Cytometer.
- The Incucyte® Phagocytosis Assay combines pHrodo® for Incucyte® reagents and integrated image-based fluorescent measurements in a simple mix-and-read protocol.
  - The iQue® Human ADCP Kit measures co-localization between live target cells and CD14+ effector cells to provide a readout for ADCP response.
  - These data exemplify that live-cell analysis, alongside advanced flow cytometry, is a powerful tool for quantitative morphological and functional assessment of phagocytosis, which is amenable to screening for therapeutic agents.

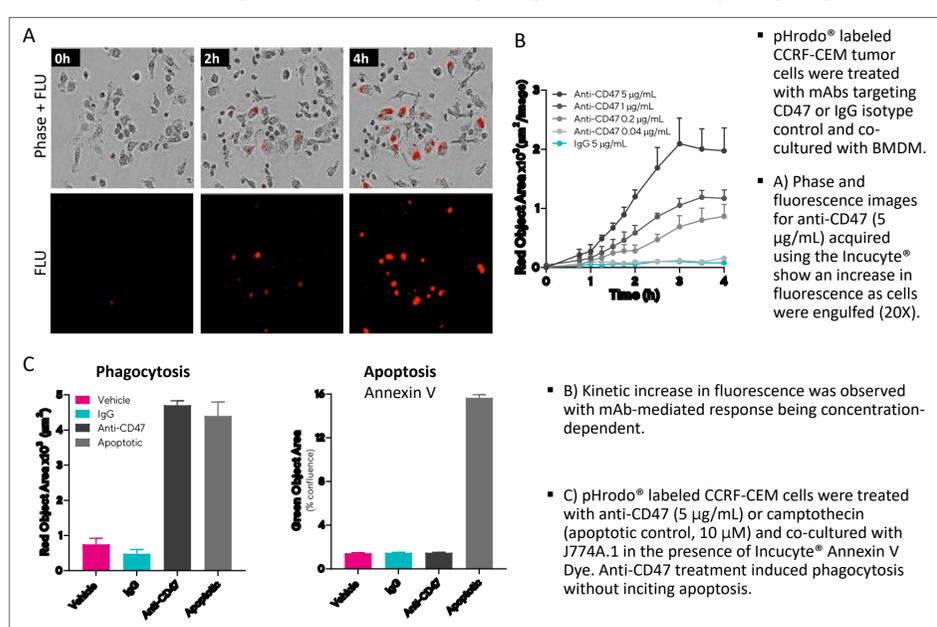
### Visualization & quantification of phagocytosis in real-time



### Phagocytosis is cell type- & Bioparticle® density-dependent

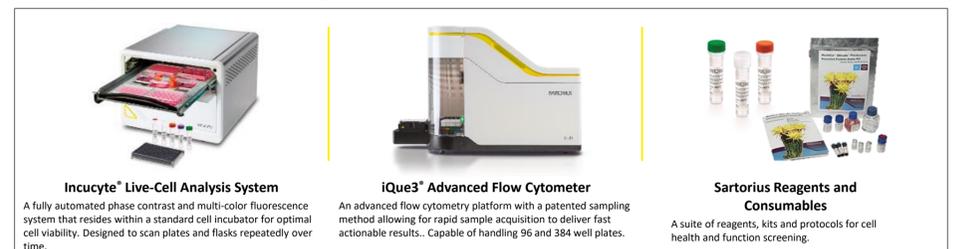


### Anti-CD47 mAb promotes macrophage-mediated phagocytosis

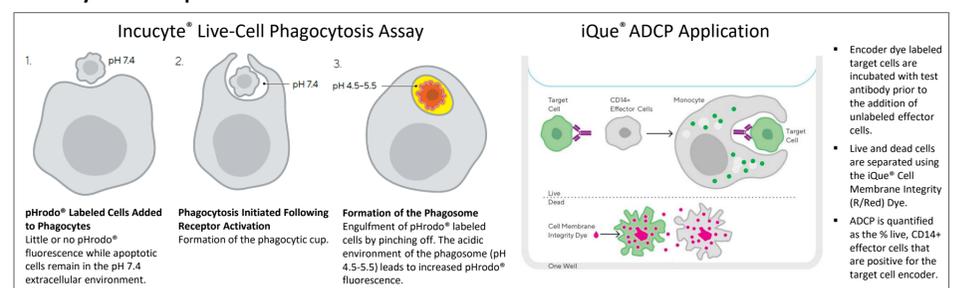


All data presented as Mean  $\pm$  SEM, N = 3 – 6 replicates.

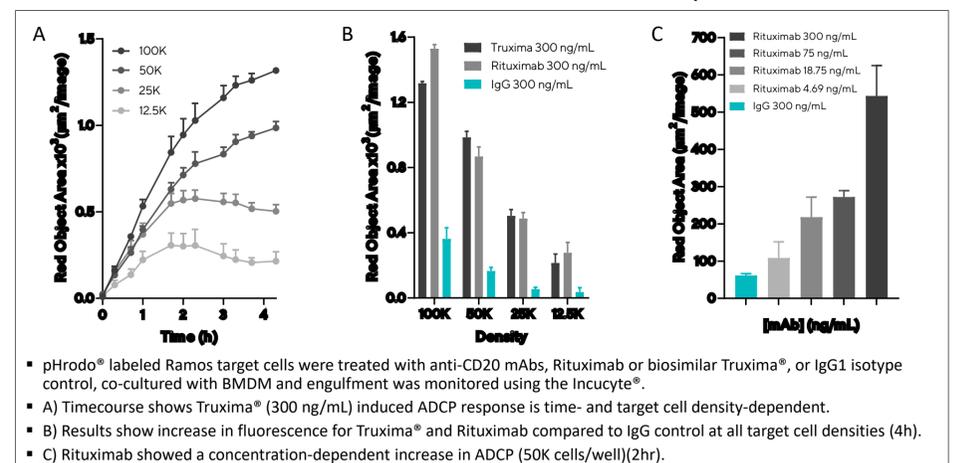
### Incucyte® & iQue3® Systems



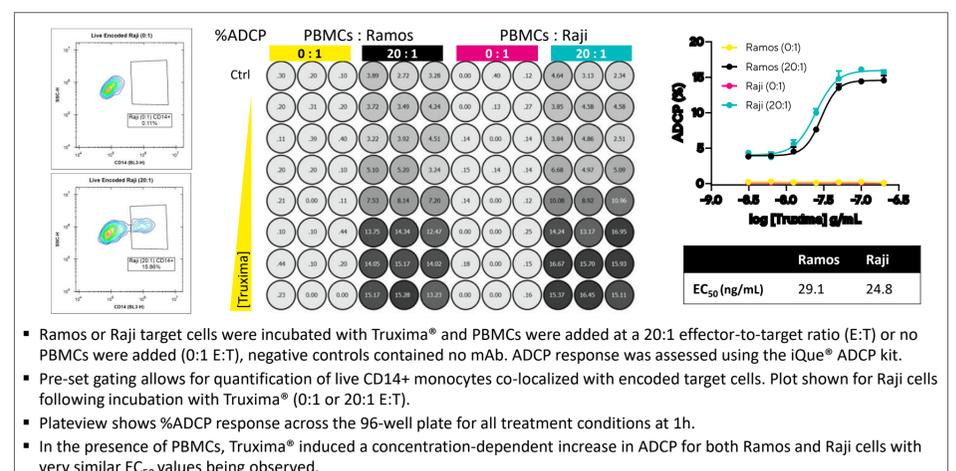
### Assay Principles



### Clinical anti-CD20 mAbs Truxima® & Rituximab promote ADCP



### ADCP increases with Truxima® concentration in B cells



### Differential ADCP response of polarized macrophages

