



On-Bead Antibody Conjugation and Purification : Magne[®] Protein A/G

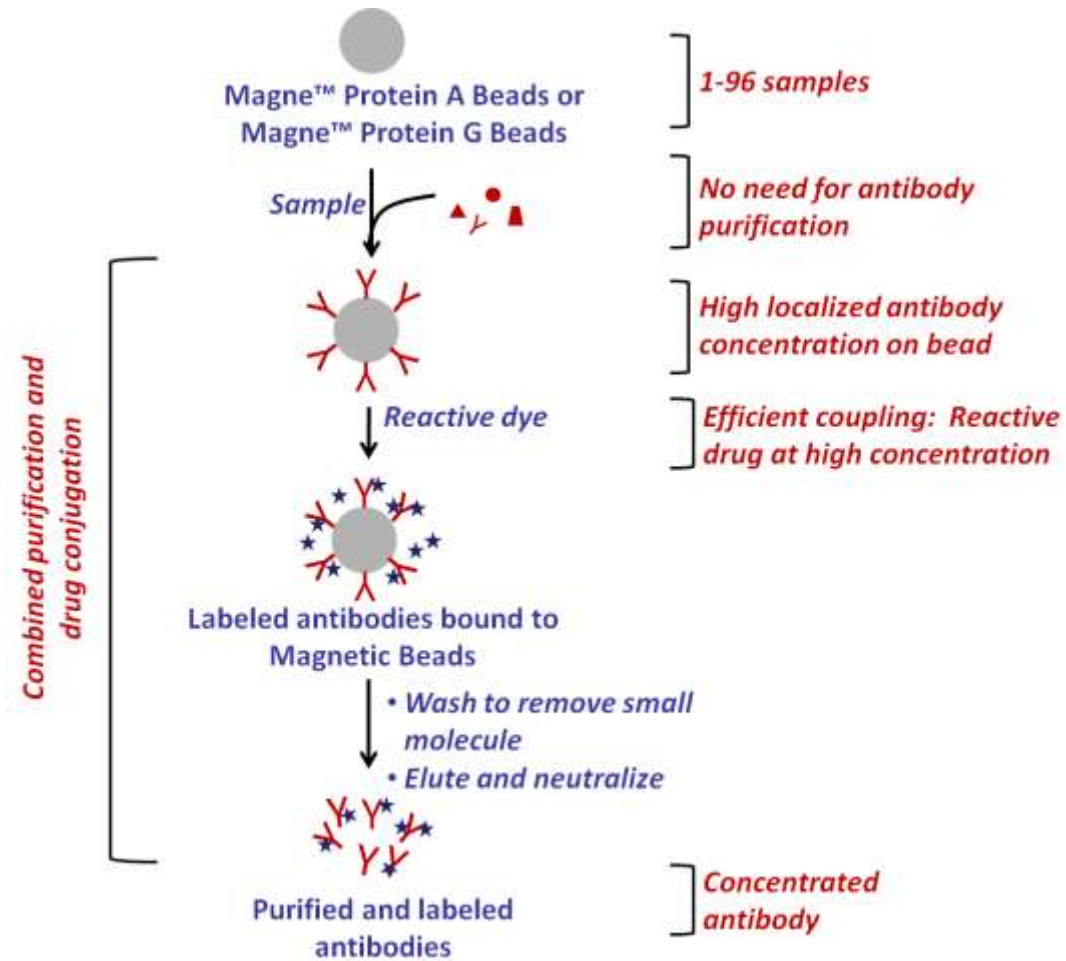
Mourad FERHAT, PhD

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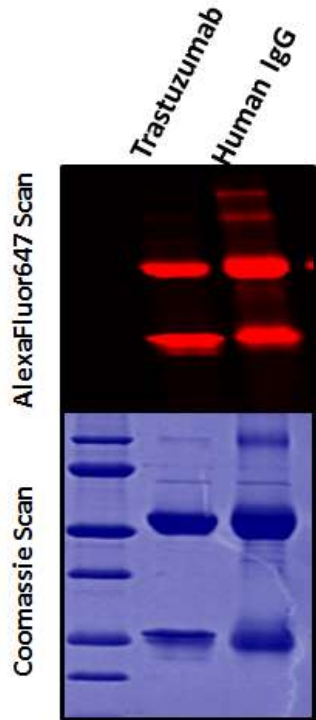


Principe de la capture/conjugaison d'anticorps

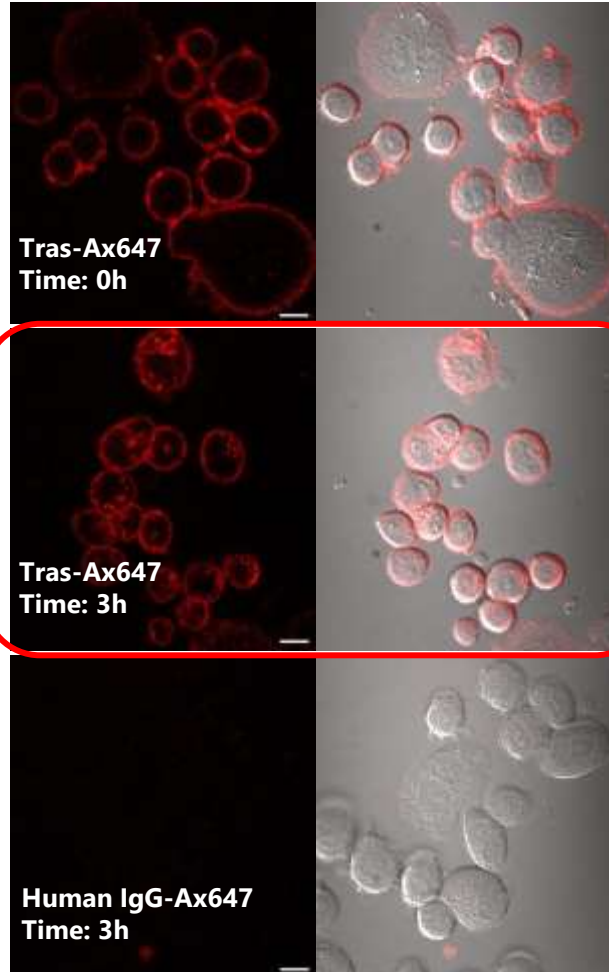


Application au Trastuzumab, suivi de l'internalisation

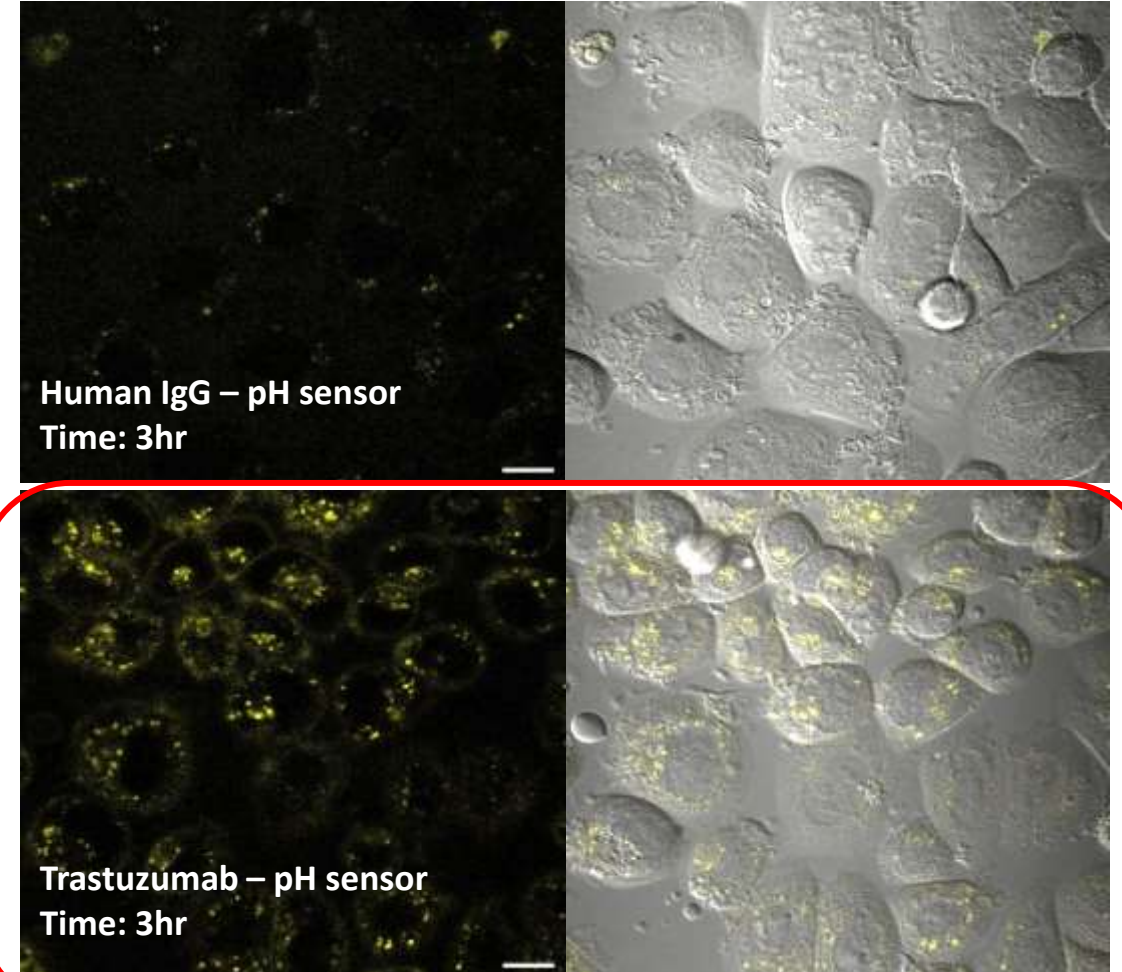
Suivi de l'internalisation



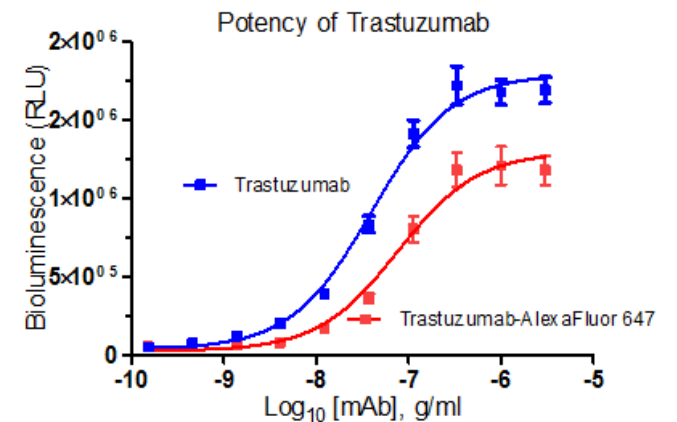
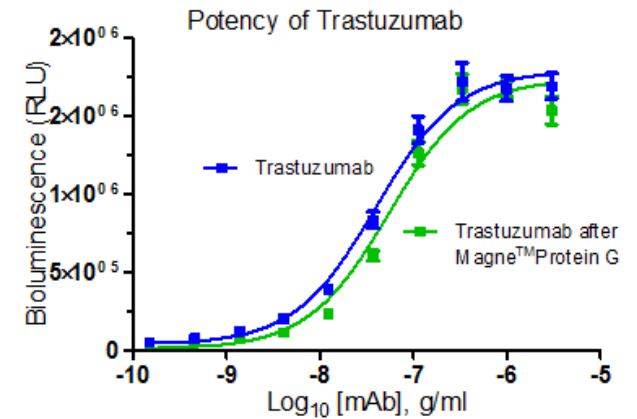
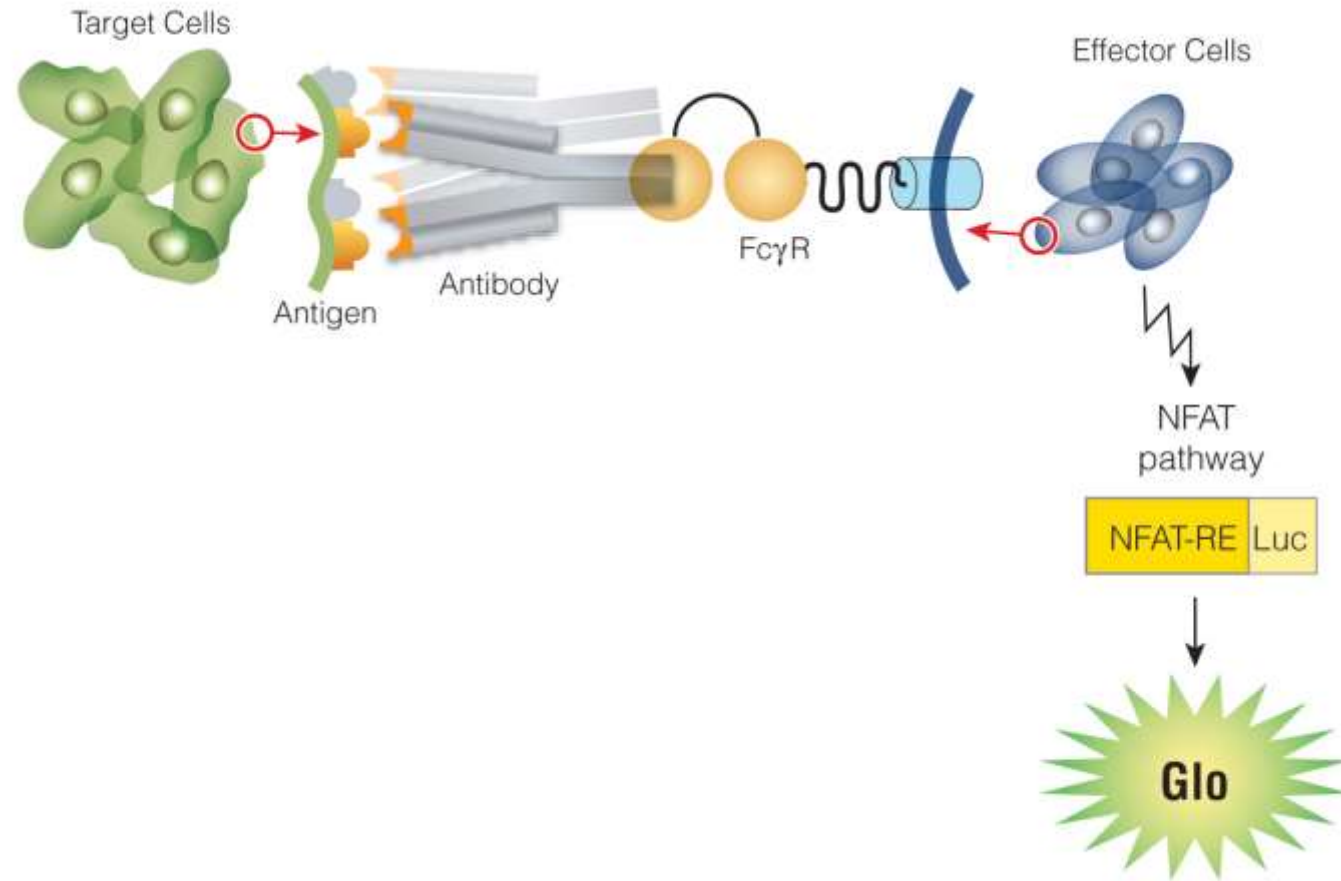
Marquage : AlexaFluor® 647



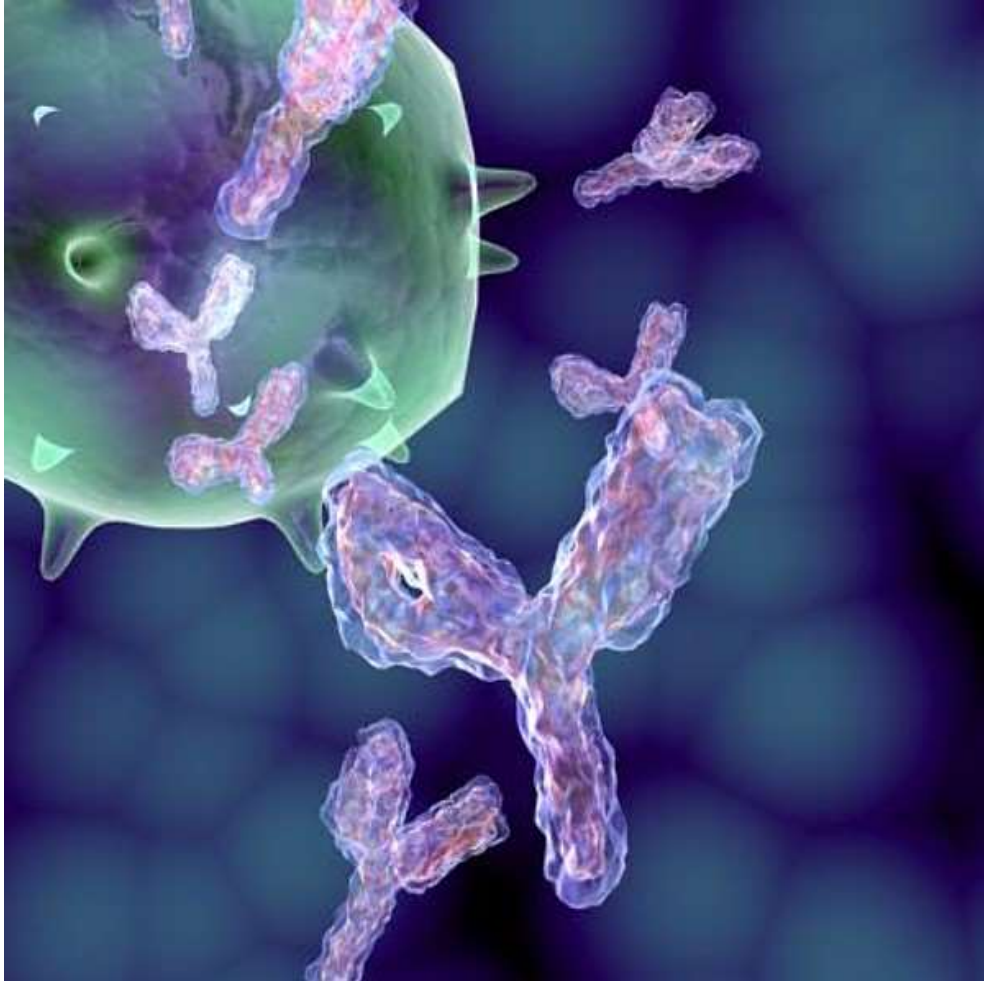
Marqueur d'internalisation : pH reactive dye, Promega



Test d'activité : ADCC Reporter Bioassay



Etude des anticorps : la gamme Promega



Antibody purification

- . Magne[®] Protein A/G
- . Streptavidin MagneSphere[®]

Cell Based Bioassays

- . Fc Receptor : ADCC/ADCP
- . Immune checkpoints : PD1/PDL1, Ox40, 4-1BB, GITR ...
- . T Cell Activation (Bispecific Antibodies)

Antibody internalization

- . pH Reactive dye

Antibody specific proteases

- . IdeS/IdeZ proteases

Poster : Session 2 - Purification et transformation

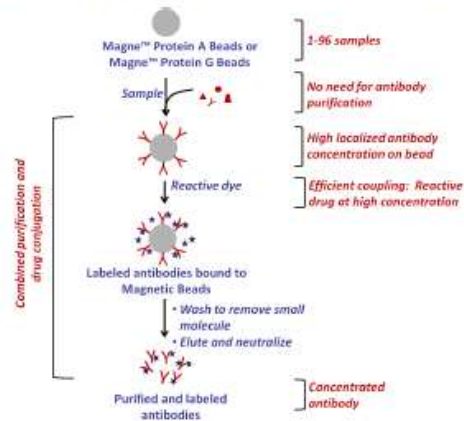
On-Bead Antibody Conjugation using High Capacity Magnetic Protein A and Magnetic Protein G Beads

Nidhi Nath, Richard Somberg, Becky Godat, H el ene Benink , Denise Garvin, Zhi-Jie Jey Cheng , Cesear Corona and Marjeta Urh
 Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711. E-mail: nidhi.nath@promega.com



1. On-Bead Antibody Conjugation

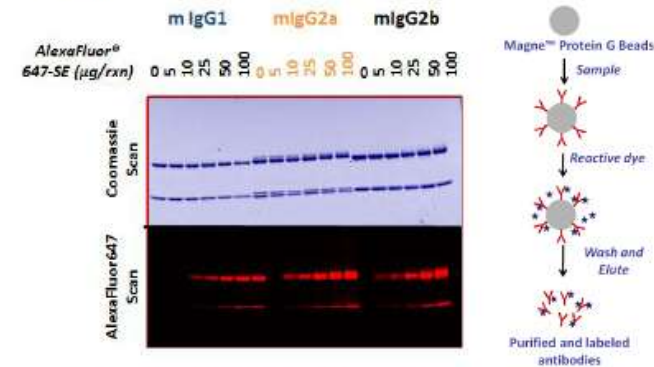
Advantages of using high capacity Magne™ Protein A and Magne™ Protein G Beads



- No need for purified antibody before conjugation
- Efficiently label dilute antibody from small –medium sample volume
- Label multiple samples (1-96) in parallel.
- Eluted labeled antibody is compatible with downstream application (Ex:

4. Antibody Purification and Conjugation through Lysines

- Three different mouse antibody isotypes from cell-media were captured on the beads.
- AlexaFluor® 647 was conjugated to the antibody using succinimidyl ester chemistry

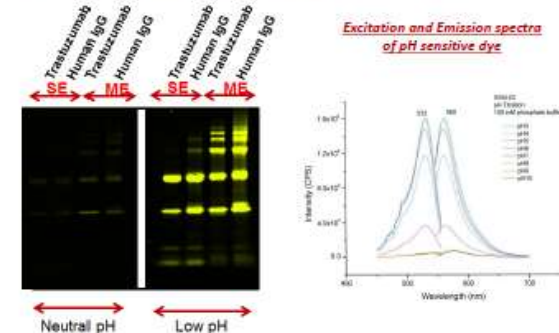


- Antibody labeling directly from cell media can be done
- AlexaFluor®647 gel scan shows increasing labeling with increase in dye amount
- Coomassie gel scan of same gel shows excellent antibody recovery

7. On-bead Conjugation of Antibodies with pH sensitive dye

- Labeling antibody with dyes that become fluorescent only upon exposure to low pH in lysosomes. This approach will enable large scale screening of internalizing antibodies suitable for ADC applications

SDS-PAGE Gel image of pH sensitive labeled antibody



- Trastuzumab and Human IgG were conjugated with pH sensitive dye through Lysines (SE) and through reduced Cysteines (ME)
- Gel image in water and at low pH show that dye conjugated to antibody retains its pH sensitive fluorescent properties