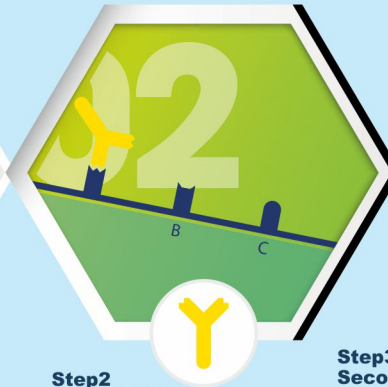
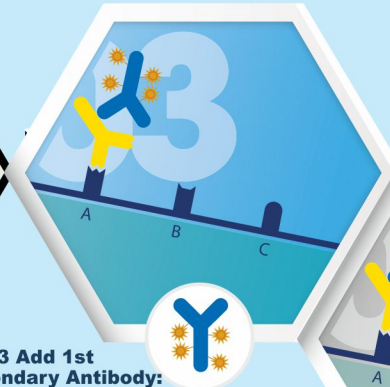


Step 1 Block:
5% Normal Donkey
Serum to block

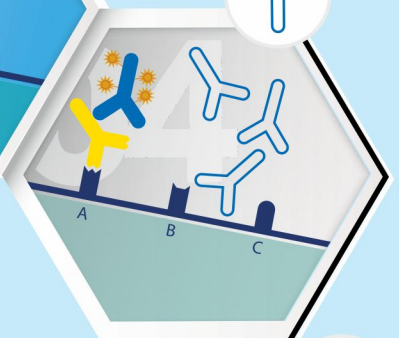


Step 2 Add 1st Primary Antibody:
Goat Anti-Antigen A

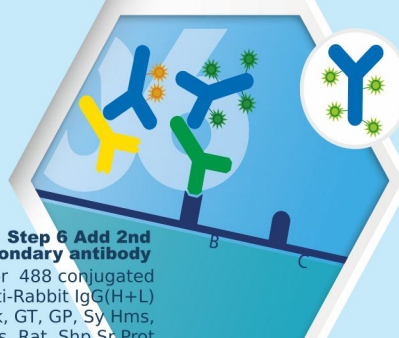


Step 3 Add 1st Secondary Antibody:
Rhodamine Red™-x
conjugated Donkey Anti-Goat
IgG (H+L)(min X Ck, GP, Sy Hms,
Hrs, Hu, Ms, Rb, Rat Sr Prot).

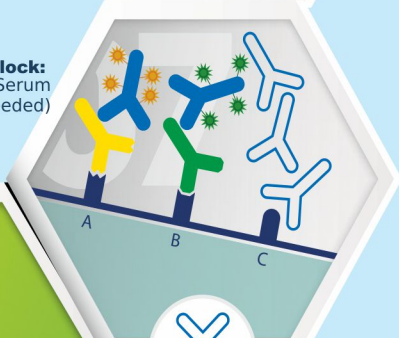
Step 4 Block:
5% Normal Donkey
Serum to block
(if needed)



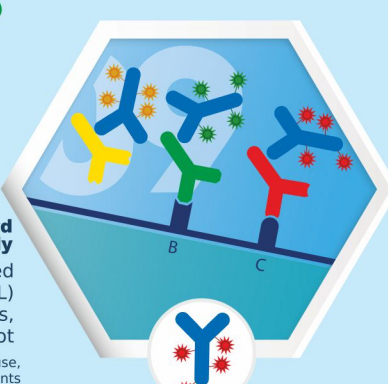
Step 5 Add 2nd primary antibody
Rabbit Anti-Antigen B



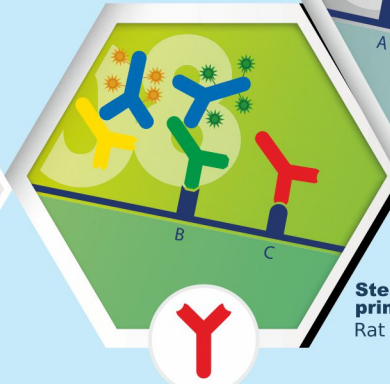
Step 6 Add 2nd secondary antibody
Alexa Fluor 488 conjugated
Donkey Anti-Rabbit IgG(H+L)
(min X Bov, Ck, GT, GP, Sy Hms,
Hrs, Hu, Ms, Rat, Shp Sr Prot



Step 7 Block:
5% Normal Donkey Serum
to Block (if needed)



Step 9 Add 3rd secondary antibody
Alexa Fluor 647 Conjugated
Donkey Anti-Rat IgG (H+L)
min X Bov, Ck, **Gt**, Gp, Sy Hms,
Hrs, Hu, **Ms**, **Rb**, Shp Sr Prot
minimal cross-reactivity with Goat, Mouse,
and Rabbit serum proteins prevents
unwanted reactions



Step 8 Add 3rd primary antibody
Rat Anti-Antigen C

Guide to Multiple Labeling with Secondary Antibodies

