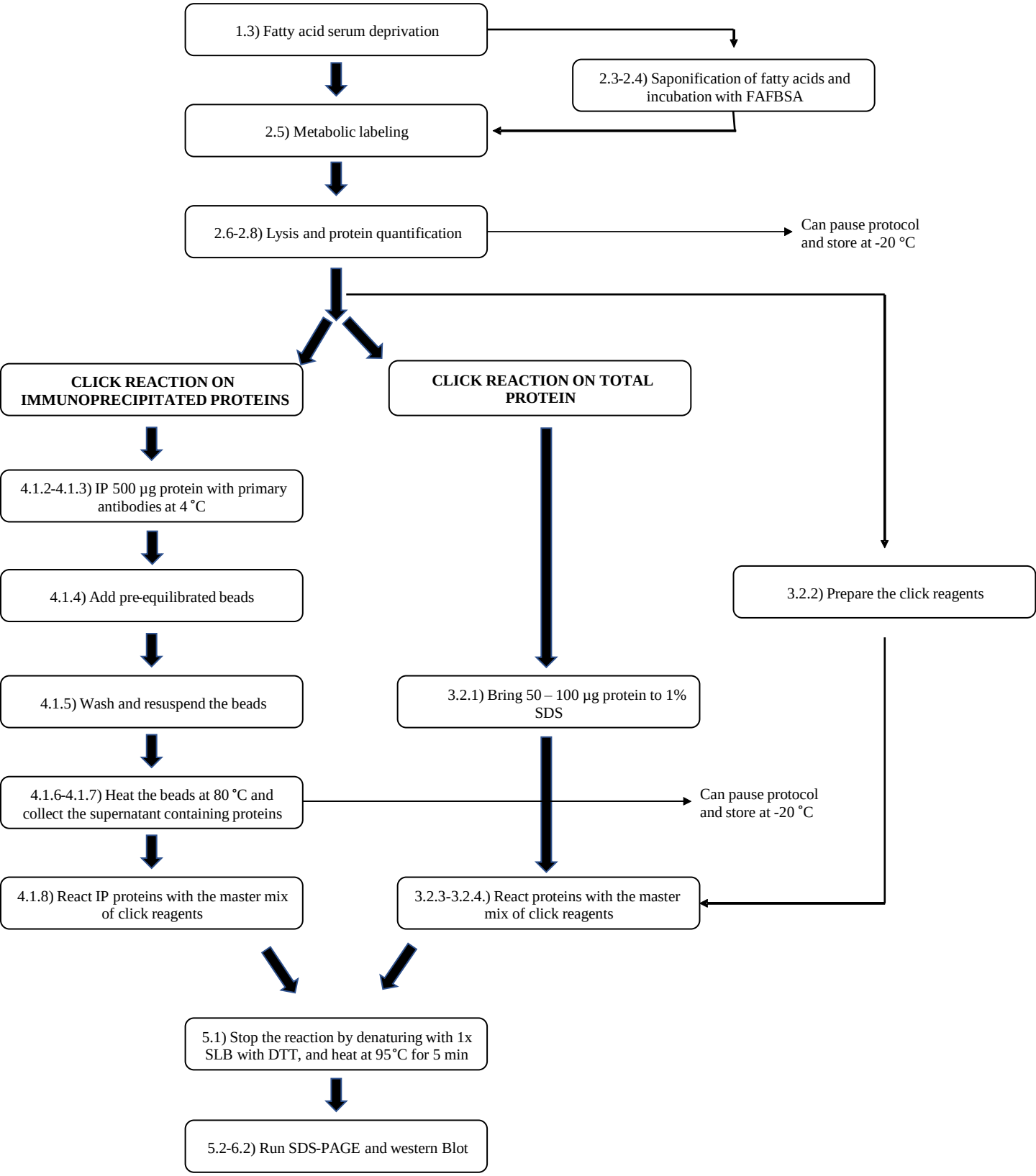
1.3) Fatty acid serum deprivation
2.3-2.4) Saponification of fatty acids and incubation with FAFBSA
2.5) Metabolic labeling
2.6-2.8) Lysis and protein quantification
Can pause protocol and store at -20 °C
CLICK REACTION ON IMMUNOPRECIPITATED PROTEINS
CLICK REACTION ON TOTAL PROTEIN
4.1.2-4.1.3) IP 500 µg protein with primary antibodies at 4 ˚C
4.1.4) Add pre-equilibrated beads
4.1.5) Wash and resuspend the beads
3.2.2) Prepare the click reagents
3.2.1) Bring 50 – 100 µg protein to 1% SDS
4.1.6-4.1.7) Heat the beads at 80 ˚C and collect the supernatant containing proteins
Can pause protocol and store at -20 ˚C
4.1.8) React IP proteins with the master mix of click reagents
3.2.3-3.2.4.) React proteins with the master mix of click reagents
5.1) Stop the reaction by denaturing with 1x SLB with DTT, and heat at 95˚C for 5 min
5.2-6.2) Run SDS-PAGE and western Blot