Sandwich ELISA steps A sandwich ELSIA gets its name from the 'sandwich' that is formed with two antibodies and an antigen during the process.Step 8: Wash away unbound detection antibody Step 9: Apply substrate for chemical colorimetric or chemiluminiscent reactions, or apply incident light for fluorescent reactions, and quantify the signal Depending on the ELISA method used, the amount of fluorescence, luminescence, or intensity of the color indicates how much target antigen was captured from the sample.Step 7: Incubate with detection antibody The detection antibody in an ELISA is conjugated to a certain label such as a flurophore (for fluorimetry), or an enzyme (for colorimetric detection or chemiluminescence).Step 2: Wash off any un-adsorbed capture protein from the well surface Washing steps with detergents reduce off-target hydrophobic interactions between proteins and remove unbound capture proteins from the plate.Proteins such as Bovine Serum Albumin (BSA), Casein, or aprotinin are commonly used to block in an ELISA assay.Step 6: Wash away the incubation fluid This is a crucial washing step that often requires multiple washes to ensure that any unbound antigen is washed away.These proteins will adsorb to the plate, preventing the target protein from accessing these sites later on. Lowering the incidence of non-specific binding in this way results in lower .background noise