Low/No signal

Possible Causes	What can you do?
Over fixation	Reduce fixation time. Perform antien retrieval to unmask epitopes.
Insufficient fixation	Increase fixation time or try other fixative.
Fixation process delayed	Fix immediately as tissue is extracted.
Permeabilization issue	"For nuclear/cytoplasmic proteins, add permeabilization agent (eg Triton X-100, Saponin) in blocking and antibody incubation buffer. For membrane/tight junction proteins, avoid permeabilization agent."
Primary antibodies not suitable for IHC	Choose an IHC-validated primary antibodies.
Wrong secondary antibody used	Make sure that primary and secondary antibodies match one another.
Low expression of protein in tissue samples	Use signal amplification methods (eg: HRP Polymer ARG80982, ARG80967, ARG80966)
Insufficient deparafinization	Make sure that parafin is removed completely before staining.

Problem with morphology

Possible Causes	What can you do?
Antigen retrieval too harsh	Optimize retrieval steps to give the best morphology.
Tissue sections peeled off slide	"Dry samples for 2-4 hours at 60°C. Tissue with high lipid content (eg breast tissues) should be dried for longer time."
Sectioning issue	Cut thinner slides for better resolution: 3-5 µm. Use a new/sharper blade.
Autolysis has occurred	"Fix samples as soon as possible. Choose other fixatives to accelerate penetration. Fixative perfusion might be necessary for larger tissues."

High background

Possible Causes	What can you do?
Insufficient blocking	Select appropriate serum as blocking buffer. Blocking for 1 hour at room temperature.
Interference from endogenous enzymes	Perform H2O2 or Levamisole quenching.
Non-specific binding from primary antibodies	Dilute primary or secondary antibody. Choose another IHC-validated primary antibodies.
Inadequate washing	"Increase washing cycles/time. Increase salt/detergent concentration for stronger washes."
Non-specific binding from secondary antibodies	Perform secondary antibody incubation only. Use pre-adsorbed secondary antibody.
Non-specific binding from chromogen	Perform chromogen incubation only. Use other chromogen if necessary.
Interference from secondary antibody in multicolor staining	Make sure that the fluorochrome does not overlap with one another.
Autofluorescence issue	Make sure that there is no endogenous background caused by tissue itself. Check under fluorescence microscope prior to staining to identify autofluorescence.