Define the objectives of IHC

Gather information on the type and duration of fixative

Search for an appropriate antibody

Test one dilution of primary Ab with different AR methods, and without AR

Staining not present

Staining present

Increase the concentration of Ab
(Decrease dilution) and repeat step 4

Staining not present

Try incubating the section overnight
with primary antibody

Staining not present

Antibody is unlikely to work
Try a different antibody

Evaluate for appropriate AR

Try various dilutions of primary
antibody to determine the optimal
dilution

Stain using a polymer-based and
at least one non-polymer based system

Pick the optimal system
and stain a few samples to check specificity

Stain the remaining samples

The truth is what you see on the slide and not what you were expecting to see
(When appropriate controls are in place)

Figure 16. Method optimization for immunohistochemical staining with a new antibody.