In the case of disulfide-bonded proteins, renaturation buffer must promote disulfide-bond formation (oxidation). The most common methods used to promote oxidation during refolding are:

1. Air oxidation . Although, the air oxidation in the presence of trace amounts of metal ions is simple and inexpensive, renaturation rates and yields can be low.

2. Addition of a mixture of the reduced (RS-) and oxidized (RSSR) forms of low molecular weight thiol reagents usually provides the appropriate redox potential to allow formation and reshuffling of disulfides.
The most common oxido-shuffling reagents are reduced and oxidized glutathione (GSH/GSSG) but the pairs cysteine/cystine, cysteamine/cystamine DTT/GSSG have also been utilized. Molar ratio of reduced to oxidized thiol of 3/1 to 1/1 and total thiol concentrations between 5-15 mM have been found to be optimal.
A disadvantage of the oxido-shuffling system is the high cost of some of the reagents, particulary glutathione.

3. Formation of mixed disulfides between oxidized glutatione and reduced protein before renaturation. Disulfide bond formation is then promoted by adding catalytic amounts of a reducing agent.

The advantages of this process are that a mixed disulfide increases the solubility of the protein during refolding and reduce the incorrect disulfide bond formation.