

Claims

1. A method of single-stranded RNA purification comprising the steps
 - applying a sample containing a mixture of double-stranded RNA and single-stranded RNA to a solid phase, the solid phase comprising a surface bearing primary amino groups on its surface, at a pH value of less than pH 8.5 sufficient to bind at least predominantly the single-stranded RNA,
 - eluting the single-stranded RNA from the surface of the solid phase by exposing the surface of the solid phase to an ascending pH gradient starting at a pH not lower than the pH used for applying the sample.
2. The method of claim 1 wherein after applying the sample and prior to eluting of the single-stranded RNA at least one step of washing of the solid phase is provided with a washing buffer having a higher ionic strength compared to an elution buffer used for eluting of the single-stranded RNA.
3. The method of claim 2 wherein at least one step of washing is provided to reduce the higher ionic strength.
4. The method of any one of the claims 1 to 3 wherein after applying the sample and prior to eluting of the single-stranded RNA at least one washing step is provided with a washing buffer having an increased pH which keeps single-stranded RNA adsorbed to the solid phase and desorbs remaining double-stranded RNA.
5. The method of any one of the claims 2 to 4 wherein the ionic strength of the washing buffer is in the range of 0.5 M to 12 M, or 1.0 M to 10 M, or 2.0 M to 8.0 M, or 4.0 M to 6.0 M higher as compared to the molarity of salt required to elute the single-stranded RNA.
6. The method of any one of the claims 2 to 5 wherein the ionic strength of the washing buffer is adjusted with a concentration of a chaotropic salt, in particular selected from the group consisting of guanidinium salts, thiocyanates, perchlorates and combinations thereof.
7. The method of any one of the claims 1 to 6 wherein eluting of the single-stranded RNA from the surface of the solid phase occurs by means of an elution

buffer having a pH in a pH range of pH 7.5 to pH 12.0, or pH 8.0 to pH 11.5, or pH 8.5 to pH 11, or pH 9.0 to pH 10.5.

8. The method of any one of the claims 1 to 7 wherein the ascending pH gradient comprises a pH range of pH 8.5 to pH 9.0.
9. The method of any one of the claims 1 to 8 wherein a chelating agent is present in the essentially aqueous mixture, in an environment of the surface of the solid phase prior to the contacting of the aqueous mixture, in a buffer for eluting the single-stranded RNA from the surface of the solid phase and/or in a separate buffer employed between the steps of applying the sample to the the solid phase and/or eluting the single-stranded RNA from the surface of the solid phase.
10. The method of claim 9 wherein the chelating agents are independently selected from the group consisting of ethylenediaminetetraacetic acid (EDTA), a salt of citric acid, phosphoric acid, or ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), tris(2-aminoethyl)-amine (TREN), and a mixture thereof.
11. The method of any one of the claims 1 to 10 wherein the single-stranded RNA is of size in the range of 1000 bases to 25,000 bases.
12. Use of a solid phase comprising a surface bearing primary amino groups on its surface for single-stranded RNA purification, wherein single-stranded RNA is purified from double-stranded RNA by means of an increasing pH, using an ascending pH gradient starting at a pH not lower than the pH used for applying the sample of single-stranded RNA.