DNase I treatment of virus supernatant.

Any source of DNase I will work.

We use our reconstituted stock DNase I, Lyophilized bovine pancreas 6.110 u/mg 5mg 14365 from AFFYMETRIX made up at 1u/µl

Digestion is usually done at 70 Units/ml of virus supernatant. If the highly purified RQ1 DNase (Promega)is used, 20 Units/ml of viral supernatant is sufficient.

1 typical reaction using DNase I.

870 ul viral supernatant,

100 ul of 10X RQ1 buffer,

add 70 units of DNase I, (1 Unit/ul)

incubate at 37 deg Celsius for 2 hour.

Viral supernatant is ready for infection and qPCR assay.

For control, 1 set of infection need to be boiled at 95 deg Celsius for 10 mins. This set should not have any Reverse transcripts.

Note 10X RQ1 buffer :

400 mM Tris.HCl pH8.0, 100 mM $MgSO_4$ and 10 mM $CaCl_2$.

Filter sterilise through 0.22 micron filter.