


## Troubleshooting Procedures

### I. Factors that may affect all High Pure kits

If you get...	Then, the cause may be...	And you should...
Low nucleic acid yield or purity	Kit stored in less than optimal conditions	▶ Store all High Pure kits at 15 to 25°C as soon as they arrive.
	Buffers or other reagents exposed to conditions that lessened their effectiveness	<ul style="list-style-type: none"> <li>▶ Store all buffers at 15 to 25°C.</li> <li>▶ If the temperature in your laboratory is lowered overnight, warm all buffers at 37°C for 10 to 15 min.</li> <li>▶ After any lyophilized reagent is reconstituted, aliquot it and store the aliquots at either 2 to 8°C or –15 to –25°C (as directed in kit package insert).</li> <li>▶ Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.</li> <li>▶ Close all wash buffer bottles tightly to prevent ethanol evaporation during storage.</li> </ul>
	Ethanol not added to Wash Buffer	<ul style="list-style-type: none"> <li>▶ Add absolute ethanol to all Wash Buffers before using (as indicated in kit package insert).</li> <li>▶ After adding ethanol, mix the Wash Buffer well and store at 15 to 25°C.</li> <li>▶ Always mark the Wash Buffer vial to indicate whether ethanol has been added or not.</li> </ul>
Reagents and sample not completely mixed	▶ Always mix the sample tube well after addition of each reagent.	
Poor elution of nucleic acids with water	Water has the wrong pH	▶ If you use double dist. water to elute nucleic acids from Filter Tube, be sure the water has the same pH as the Elution Buffer supplied in the kit.
	Used a buffer from a different High Pure Kit.	<ul style="list-style-type: none"> <li>▶ The Elution Buffer from the different High Pure Kits have different pHs depending on the nucleic acid being purified. Swapping buffers may cause low yields.</li> </ul> <p> <i>Recovery can be improved by incubating the column filter with elution buffer for 1 to 5 min after addition of elution buffer.</i></p>
Incomplete or no restriction enzyme cleavage of product	Glass fibers, which coeluted with the nucleic acid, inhibit enzymes	<ul style="list-style-type: none"> <li>▶ 1. Remove High Pure Filter Tube from tube containing eluted sample and spin sample tube for 1 min at maximum speed.</li> <li>▶ 2. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.</li> </ul>





## I. Factors that may affect all High Pure kits, continued

If you get...	Then, the cause may be...	And you should...
Absorbance ( $A_{260}$ ) reading of product too high	Glass fibers, which coeluted with the nucleic acid, scatter light	▶ See suggestions under “Incomplete or no restriction enzyme cleavage of product” above.
Samples “pop” out of wells in agarose gel	Eluate contains ethanol (from the Wash Buffer)	▶ 1. After the last wash step, do not let the flowthrough (used Wash Buffer) touch the bottom of the High Pure Filter Tube. ▶ 2. Empty Collection Tube, reinsert Filter Tube in emptied Collection Tube, and recentrifuge for 30 s.

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## II. Factors that may affect the High Pure PCR Template Preparation Kit

If you get...	Then, the cause may be...	And you should...
Low yield from any starting material	Proteinase K not completely solubilized	To solubilize the lyophilized Proteinase K completely: <ul style="list-style-type: none"> <li>▶ 1. Pipette 4.5 ml of double dist. <math>H_2O</math> into the glass vial containing lyophilized Proteinase K.</li> <li>▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>▶ 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at <math>-15</math> to <math>-25^\circ C</math>.</li> </ul> <p> <i>Reconstituted Proteinase K is stable for 12 months when stored properly.</i></p>
	Incomplete lysis	<ul style="list-style-type: none"> <li>▶ After adding Proteinase K to sample, mix immediately.</li> <li>▶ Always mix lysate well with isopropanol before adding it to the High Pure Filter Tube.</li> </ul>
Low yield from tissue	Incomplete Proteinase K digestion	<ul style="list-style-type: none"> <li>▶ Cut tissue into small pieces before digestion and lysis.</li> <li>▶ Increase incubation time with Proteinase K (Step 1 of Protocol Vb) in either of two ways:                             <ul style="list-style-type: none"> <li>▶ Incubate tissue with Proteinase K overnight.</li> <li>▶ Incubate with Proteinase K for 3 – 4 h, then add a fresh aliquot of Proteinase K (30 <math>\mu l</math>) and incubate another 1 – 2 h.</li> </ul> </li> </ul> <p> <i>To accommodate increased volume (sample + enzyme), use 230 <math>\mu l</math> Binding Buffer instead of 200 <math>\mu l</math> in Step 2 of Protocol Vb.</i></p>
Low yield from bacteria or yeast	Cells not lysed efficiently with lysozyme (bacteria) or lyticase (yeast)	<ul style="list-style-type: none"> <li>▶ Make sure your cells can be lysed by lysozyme or lyticase. The kit package insert lists some recommended strains (which are known to be lysed by the enzymes).</li> <li>▶ Use alternative lysis procedures, such as mechanical grinding, vortexing with glass beads, boiling, or repeated freeze-thaw.</li> </ul>



## II. Factors that may affect the High Pure PCR Template Preparation Kit, *continued*

If you get...	Then, the cause may be...	And you should...
Degraded DNA from tissue samples	Nuclease activity in unlysed tissue	<ul style="list-style-type: none"> <li>▶ Tissue should be frozen (–15 to –25°C) from the time of harvest until the lysis procedure starts.</li> <li>▶ Use only small pieces of tissue (20 – 40 mg) in the procedure, or homogenize the tissue sample.</li> </ul>
Eluate from blood samples is still slightly colored	Incomplete wash	<ul style="list-style-type: none"> <li>▶ Wash Filter Tube until flowthrough is colorless.</li> <li>▶ Repeat purification protocol by mixing 200 µl eluate with 200 µl Binding Buffer, then 100 µl isopropanol. Follow Protocol Va (page 23), starting with the application of the sample to the High Pure Filter Tube (Step 3).</li> </ul> <p style="text-align: right;"><b>!</b> <i>Omit Proteinase K digestion and 72°C incubation.</i></p>

## III. Factors that may affect the High Pure PCR Cleanup Micro Kit

If you get...	Then, the cause may be...	And you should...
Low recovery of nucleic acids in eluate	Non-optimal reagent has been used for elution	<ul style="list-style-type: none"> <li>▶ Do not use water to elute nucleic acids from Filter Tube. Alkaline pH is required for optimal elution.</li> <li>▶ Use the Elution Buffer in the kit.</li> </ul>
	Incomplete elution	Elute DNA with two volumes of Elution Buffer (>10 µl each). Be sure to centrifuge after each addition of Elution Buffer.
Concentration of DNA in the eluate is too low	Low concentrations of amplified DNA were added to the High Pure Filter Tube (in Step 1)	<p>Verify PCR result by agarose gel electrophoresis before starting purification procedure.</p> <p><b>!</b> <i>Do not use less than 10 µl Elution Buffer</i></p>
No PCR product in final eluate	No PCR product in starting material	Verify PCR result by agarose gel electrophoresis before starting purification procedure.
Short read length in sequencing reactions	Copurification of DNA binding proteins	Use the Expand High Fidelity PCR System for template synthesis.
	Copurification of primer and primer-dimer	Use Binding buffer without Binding Enhancer for purification.

#### IV. Factors that may affect the High Pure PCR Product Purification Kit





If you get...	Then, the cause may be...	And you should...
Low yield	Reagents exposed to conditions that lessened their effectiveness	▶ Store all buffers at 15 to 25°C.
	Reagent and sample not completely mixed	▶ Always mix contents of sample tube well after each reagent addition.
	Too little Binding Buffer used	▶ Make sure the ratio of PCR product to Binding Buffer is 1:5. <i>Oil overlay, wax, and gel loading dye do not interfere with the purification procedure.</i>
	Incomplete elution	▶ Elute product with two volumes of Elution Buffer (50 µl each), centrifuging after addition of each volume.
Concentration of DNA in eluate too low	Too much Elution Buffer	▶ Decrease volume of Elution Buffer. <i>Do not use &lt;50 µl.</i>
No PCR product in final eluate	No PCR Product in starting material	▶ Check PCR result by agarose gel electrophoresis before starting purification procedure.

#### V. Factors that may affect the High Pure Plasmid Isolation Kit

If you get...	Then, the cause may be...	And you should...
Low plasmid yield	Too few cells in starting material	▶ Grow <i>E. coli</i> to an absorbance ( $A_{600}$ ) of 1.0 – 1.9 before harvest.
	Incomplete cell lysis	▶ Be sure the <i>E. coli</i> pellet is completely resuspended in Suspension Buffer.
		▶ Make sure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer). ▶ Make sure a cloudy white precipitate forms when Binding Buffer is added to lysate. The precipitate should pellet completely during centrifugation.
Plasmid is degraded or no plasmid obtained	Lysate did not bind completely to High Pure Filter Tube	▶ Pre-equilibrate the glass fleece in the Filter Tube by adding 200 µl Binding Buffer to the Filter Tube before applying sample. <i>Do not centrifuge the Filter Tube after this step. Instead, apply the sample and centrifuge as directed in Step 6 of the protocol.</i>
Plasmid is degraded or no plasmid obtained	High levels of nuclease activity	▶ Use optional Wash Buffer I (Step 7 of protocol) to eliminate nuclease activity in <i>E. coli</i> strains with high levels of nuclease (for example, HB 101).



## V. Factors that may affect the High Pure Plasmid Isolation Kit, continued

If you get...	Then, the cause may be...	And you should...
RNA present in final product	RNase not completely dissolved	<p>To solubilize the lyophilized RNase completely:</p> <ul style="list-style-type: none"> <li>▶ 1. Pipette 1 ml of Suspension Buffer into the glass vial containing lyophilized RNase.</li> <li>▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>▶ 3. Transfer all the reconstituted RNase back into the Suspension Buffer vial and mix thoroughly.</li> <li>▶ 4. Mark the reconstituted mixture (enzyme + buffer) with the date of reconstitution, and store at 2 to 8°C.</li> </ul> <p> <i>Reconstituted mixture is stable for 6 months when stored properly.</i></p>
	Too many cells in starting material	<ul style="list-style-type: none"> <li>▶ Do not use more than 4 ml of an overnight <i>E. coli</i> culture as starting material.</li> </ul>
Genomic DNA present in final product	Genomic DNA sheared during lysis step	<ul style="list-style-type: none"> <li>▶ At Step 3 of the protocol, mix the Lysis Buffer and sample by gentle inversion of the tube.</li> </ul> <p> <i>Do not vortex!</i></p>
Additional band running slightly faster than supercoiled plasmid is seen on gels	Denatured plasmid in final product	<ul style="list-style-type: none"> <li>▶ Reduce the incubation time during Step 3 (lysis step) of the protocol.</li> </ul>





## VI. Factors that may affect the High Pure RNA Isolation Kit

If you get...	Then, the cause may be...	And you should...
No RNA	High levels of RNase activity	<ul style="list-style-type: none"> <li>▶ Be careful to create an RNase-free working environment.</li> <li>▶ Process starting material immediately or store it at -80°C until it can be processed.</li> <li>▶ Use eluted RNA directly in downstream procedures or store it immediately at -80°C.</li> </ul>
	Cultured cells not completely resuspended	<ul style="list-style-type: none"> <li>▶ Resuspend cell pellet completely in PBS before starting procedure.</li> </ul>










## VI. Factors that may affect the High Pure RNA Isolation Kit,

continued

If you get...	Then, the cause may be...	And you should...
DNA present in final product	DNase not completely dissolved	To solubilize the lyophilized DNase completely: <ol style="list-style-type: none"> <li>1. Pipette 0.55 ml of Elution Buffer into the glass vial containing lyophilized DNase.</li> <li>2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</li> </ol> <p> <i>Reconstituted DNase is stable for 12 months when stored properly. To create a working DNase solution for 10 samples, mix 100 <math>\mu\text{l}</math> reconstituted DNase with 900 <math>\mu\text{l}</math> DNase Incubation Buffer.</i></p>
	DNase not evenly distributed in High Pure Filter Tube	<p> When pipetting, distribute the working DNase solution evenly over the glass fleece in the Filter Tube.</p> <p> <i>Do not stick pipette tip into the glass fleece when pipetting the DNase.</i></p>
	Too much starting material	<p> Do not use more than <math>10^6</math> cultured cells, 0.5 ml whole blood, <math>10^8</math> yeast, or <math>10^9</math> bacteria.</p>

## VII. Factors that may affect the High Pure RNA Tissue Kit

If you get...	Then, the cause may be...	And you should...
Low nucleic acid yield or purity	Tissue stored and handled in less than optimal conditions	<p> Use fresh tissue and disrupt immediately or flash frozen tissue stored at <math>-60^{\circ}\text{C}</math> or below. Frozen tissue should not be allowed to thaw during handling prior to disruption in Lysis/Binding Buffer.</p>
	Ethanol not added to the lysate	<p> Addition of 0.5 volume of absolute ethanol to the lysate is necessary to promote selective binding of RNA to the glass fibers.</p>
	High levels of RNase activity	<p> Be careful to create an RNase-free working environment.</p> <p> Process starting material immediately or store it at <math>-80^{\circ}\text{C}</math> until it can be processed.</p> <p> Use eluate RNA directly in downstream procedures or store it immediately at <math>-80^{\circ}\text{C}</math>.</p>
Tissue homogenate is viscous and difficult to pipet, low RNA yield	Insufficient disruption or homogenization	<p> Add 350 <math>\mu\text{l}</math> of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity.</p>
	Too much starting material	<p> Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.</p>



## VII. Factors that may affect the High Pure RNA Tissue Kit,

continued

If you get...	Then, the cause may be...	And you should...
Clogged filter tube	Insufficient disruption and/or homogenization	▶ <i>E.g.</i> , increase the disruption time for the rotor stator homogenizer or pass through syringe/needle additional times.
	Too much starting material	▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.
DNA contamination	Lysis/Binding Buffer not completely removed from the glass fleece	▶ Increase centrifugation time.

## VIII. Factors that may affect the High Pure FFPE RNA Micro Kit


If you get...	Then, the cause may be...	And you should...
Low RNA yield or purity	High levels of RNase activity	▶ Be careful to create an RNase-free working environment.
		▶ Process starting material immediately or store it at -80°C until it can be processed.
		▶ Use eluted RNA directly in downstream procedures or store it immediately at -80°C.
Tissue homogenate is viscous and difficult to pipet, low RNA yield	Insufficient disruption or homogenization	▶ Add 350 µl of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity.
Low yield and /or bad performance in RT-PCR	Nucleic acid is cross-linked to impurities	▶ Increase Proteinase K digestion time in step 1 of the RNA Isolation Protocol to 16 h (overnight).
	Too much starting material	▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.
Clogged filter tube	Insufficient disruption and/or homogenization	▶ <i>E.g.</i> , increase the disruption time for the rotorstator homogenizer or pass through syringe/ needle additional times.
	Too much starting material	▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.

## IX. Factors that may affect the High Pure RNA Paraffin Kit

If you get...	Then, the cause may be...	And you should...
Low RNA yield and purity	Inappropriate storage and handling of tissue	▶ Use fresh tissue and disrupt immediately or flash-frozen tissue stored at $-70^{\circ}\text{C}$ . Frozen tissue should not be allowed to thaw during handling prior to disruption in Lysis/Binding Buffer
Tissue homogenate is viscous and difficult to pipet	Insufficient disruption or homogenization	▶ Add 350 $\mu\text{l}$ of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity
	Too much starting material	▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer
Clogged filter tube	Insufficient disruption and/or homogenization	▶ Increase the disruption time for <i>e.g.</i> , the rotor stator homogenizer or pass through syringe/needle several times.
	Too much starting material	▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer

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## X. Factors that may affect the High Pure Viral RNA Kit

If you get...	Then, the cause may be...	And you should...
Low RNA yield	High levels of RNase activity	▶ See suggestions under “No RNA” in “Factors that may affect the High Pure RNA Isolation Kit” page 102.
	Carrier RNA not completely dissolved	<p>To solubilize the lyophilized Poly(A) Carrier RNA completely:</p> <ul style="list-style-type: none"> <li>▶ 1. Pipette 0.4 ml Elution Buffer into the glass vial containing lyophilized Carrier RNA.</li> <li>▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>▶ 3. Aliquot the carrier RNA mark each aliquot with the date of reconstitution, and store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</li> </ul> <p> <i>Thaw one vial with 4 <math>\mu\text{l}</math> carrier RNA and mix thoroughly with 5 ml Binding Buffer.</i></p>


## XI. Factors that may affect the High Pure Viral Nucleic Acid Kit

If you get...	Then, the cause may be...	And you should...
Low RNA yield	High levels of RNase activity	<ul style="list-style-type: none"> <li>▶ See suggestions under “No RNA” in “Factors that may affect the High Pure RNA Isolation Kit” page 102.</li> </ul>
Low DNA or RNA yield	Carrier RNA not completely dissolved	<ul style="list-style-type: none"> <li>▶ To solubilize the lyophilized Poly(A) Carrier RNA completely:                             <ol style="list-style-type: none"> <li>1. Pipette 0.5 ml Elution Buffer into the glass vial containing lyophilized Carrier RNA.</li> <li>2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>3. Aliquot the Carrier RNA, mark each aliquot with the date of reconstitution, and store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</li> </ol> </li> <li>! <i>Thaw one vial with 50 <math>\mu\text{l}</math> carrier RNA and mix thoroughly with 2.5 ml Binding Buffer.</i></li> </ul>
	Incomplete Proteinase K digestion	<ul style="list-style-type: none"> <li>▶ Be sure to dissolve the lyophilized Proteinase K completely, as follows:                             <ul style="list-style-type: none"> <li>▶ 1. Pipette 5 ml of double dist. <math>\text{H}_2\text{O}</math> into the glass vial containing lyophilized Proteinase K.</li> <li>▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>▶ 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</li> </ul> </li> <li>! <i>Reconstituted Proteinase K is stable for 12 months when stored properly.</i></li> </ul>



## XII. Factors that may affect the High Pure 16 System Viral Nucleic Acid Kit

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If you get...	Then, the cause may be...	And you should...
Low RNA yield	High levels of RNase activity	<ul style="list-style-type: none"> <li>▶ Be careful to create an RNase-free working environment.</li> <li>▶ Process starting material immediately or store it at <math>-80^{\circ}\text{C}</math> until it can be processed.</li> <li>▶ Use eluted RNA directly in downstream procedures or store it immediately at <math>-80^{\circ}\text{C}</math>.</li> </ul>
	Carrier RNA not completely dissolved	▶ See suggestions in the package insert.
	Incomplete Proteinase K digestion	<p>Be sure to dissolve the lyophilized Proteinase K completely, as follows:</p> <ul style="list-style-type: none"> <li>▶ 1. Pipet 5 ml of Elution Buffer into the glass vial containing lyophilized Proteinase K.</li> <li>▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</li> <li>▶ 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</li> </ul> <p> <i>Reconstituted Proteinase K is stable for 12 months when stored properly.</i></p>