IQeasy™ plus Blood RNA Extraction Mini Kit Handbook

Instruction Manual

For purification of total RNA from Blood samples

Cat. No. 17331 | 50 Columns

Ver 1.0

iNtRON Biotechnology, Inc.

www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com

Contents

Kit Contents

Label	Description	Contain
Buffer RBL	Pre-lysis Buffer	50 ml x 4 bottles
Buffer RLB[1]	Lysis Buffer	22 ml
Buffer RWA	Washing Buffer A	40 ml
Buffer RWB (concentrate)[2]	Washing Buffer B	10 ml
Buffer RE	Elution Buffer	20 ml
gDNA Remover Spin Columns	gDNA Eliminator Column	50 columns
Spin Columns (Red color O-ring)	Inserted into a collection tubes (2.0ml tubes)	50 columns
Collection Tubes (2.0ml tubes)	Additionally supplied.	100 tubes

[1] BufferRLBcontainschaotropicsaltcanformhighlyreactivecompound.
[2] BufferRWBissuppliedasconcentrates.Add40ml ofethanol (96~100%)accordingtothebottlelabel beforeuse.

Storage

TheIQeasy ™ plusBloodRNAExtractionMini Kitshouldbestoreddryatroomtemperature(15~25ºC) andisstableforatleast12monthsundertheseconditions

Quality Control

InaccordancewithiNtRON"sISO-certifiedQualityManagement System, eachlot of IQeasy™ plus BloodRNAExtractionMini Kit istestedagainst predeterminedspecificationstoensureconsistent productquality.

Safety Information

Whenworkingwithchemicals, alwaysshouldwear asuitablelabcoat, disposablegloves, and protectivegoggles. Formoreinformation, pleaserequest theappropriatematerial safetydatasheets (MSDS). Donot addbleachor acidicsolutionsdirectlytothewaste. Buffer RLBandBuffer RWA containsachaotropicsalts,whichcanformhighlyreactivecompoundswhencombinedwithbleach. Ifliquidcontainingthisbufferisspilt,cleanwithsuitablelaboratorydetergentandwater.

Product Warranty and Satisfaction Guarantee

All productsundergoextensivequalitycontrol test andarewarrantedtoperformasdescribedwhen usedcorrectly. Immediatelyanyproblemsshouldbereported. Satisfactionguaranteeisconditional uponthecustomerprovidingfull detailsof theproblemtoiNtRONwithin60days, andreturningthe producttoiNtRONforexamination

Product Use Limitations

TheIQeasy ™ plusBloodRNAExtractionMini Kitisintendedforresearchuseonly.Priortousingitfor otherpurposes, theusermust validatethesystemincompliancewiththeapplicablelaw, directives, andregulations. IQeasy™ plusBloodRNAExtractionMini Kit isdeveloped, designed, andsoldfor researchpurposeonly.Theyarenottobeusedforhumanoranimal diagnosisofdiseases.Donotuse internallyorexternallyinhumansoranimals.Becareful inthehandlingoftheproducts.

Technical Assistance

At iNtRONweprideourselvesonthequalityandavailabilityof ourtechnical support. OurTechnical ServiceDepartmentsarestaffedbyexperiencedscientistswithextensivepractical andtheoretical expertiseinmolecular biologyandtheuseof iNtRONproducts. If youhaveanyquestionsor experienceanydifficultiesregardingtheIQeasy ™ plusBloodRNAExtractionMini Kit or iNtRON productsingeneral,pleasedonothesitatetocontactus.
iNtRONcustomersareamajorsourceof informationregardingadvancedorspecializedusesof our products. Thisinformationishelpful tootherscientistsaswell astotheresearchersat iNtRON. We thereforeencourageyoutocontactusifyouhaveanysuggestionsaboutproductperformanceornew applicationsandtechniques. For technical assistanceandmoreinformationpleasecall oneof the iNtRONTechnicalServiceDepartmentsorlocaldistributors.

Precautions and Safety Information

All chemicalsshouldbeconsideredaspotentiallyhazardous. Whenworkingwithchemicals, always wearasuitablelabcoatanddisposablegloves.Somebuffercontainthechaotrophicsaltwhichmaybe anirritantandcarcinogen,soappropriatesafetyapparel suchasglovesandeyeprotectionshouldbe worn.Ifaspillofthebuffersoccurs,cleanwithasuitablelaboratorydetergentandwater.
If theliquidspill containspotentiallyinfectiousagents, cleantheaffectedareafirst withlaboratory detergent andwater, thenwithasuitablelaboratorydisinfectant. Onlypersonstrainedinlaboratory techniquesandfamiliarwiththeprinciplesofgoodlaboratorypracticeshouldhandletheseproducts.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Equipment and Reagents to be Supplied by User

IQeasy™ plusBloodRNAExtractionMiniKitprovidesalmostallreagentsforextractingRNA.However, youshouldpreparesomeequipmentsandreagentsasfollowsforafast andeasyextraction. When workingwithchemicals,alwayswearasuitablelabcoat,disposablegloves,andprotectivegoggles.

IQeasy™ plusBloodRNAExtractionMiniKitprovidesalmost

- Ethanol(96~100%)
- Micro-centrifuge
- Microcentrifugetubes(1.5ml)
- Othergeneral labequipments
- β-Mercaptoethanol (14.2M)
- Vortexmixer
- Micro-pipettesandpipettetips

Description

TheIQeasy ™ plusBloodRNAExtractionMini Kitisprovideafast,easymethodforthepreparationof total cellularRNAfromupto1ml ofwholeblood.Also,TheIQeasy™plusBloodRNAExtractionMini Kitcanbeusedtopurifytotal RNAfromwholebloodandtoseparateRNAfromproteins,saltandother reactioncomponents. Contaminantsandenzymeinhibitorssuchashemoglobinandheparinare completelyremoved, leavingpurifiedRNAreadyfor useindownstreamapplications. Duringthe IQeasy™ plusBloodRNAExtractionMiniKitprocedureforpurificationofRNAfromblood,erythrocytes areselectivelylysedandleukocytesarerecoveredbycentrifugation. Theleukocytesarethenlysed usinghighlydenaturingconditionsthat immediatelyinactivateRNases, allowingtheisolationof intact RNA. RNAisboundtothesillicamembraneduringabrief centrifugationstep. Contaminantsare washedawayandtotal RNAiselutedin30 µl or moreof RNAse-freewater for direct useinany downstreamapplication.Isolatedhigh-qualityRNAfromtheIQeasy ™ plusBloodRNAExtractionMini Kit isaprerequisitefor manygeneexpressionprofilingtechniquessuchasquantitativePCRor microarrayanalysis.TheIQeasy ™ plusBloodRNAExtractionMini KitcanbeisolatedRNAfromwhole bloodin~30min. Yieldsof total RNAaretypically3~10µgfrom1ml of wholebloodfromnormal healthydonors.

Characteristics

- Isolatedhigh-qualityRNAissuitableformanygeneexpressionprofilingtechniques:
  - ✓ cDNAsynthesis
  - ✓ ReverseTranscriptasePCR(RT-PCR)
  - ✓ QuantitativePCR(qPCR,qRT-PCR)
  - ✓ Microarray
  - ✓ Northernandslotblotting,RNasenucleaseprotection
- AdvancedGxNtechnologyforrapidandefficientpurificationwithin30minoftotalRNAfrom wholebloodsample
- ChaotropicsaltinlysisbufferinactivatesimmediatelyRNasetoensureisolationofintactRNA.

Column Information

- IQeasy™ plusBloodRNAExtractionMiniKit"sSpinColumn

Column membrane[1]	Silica-based membrane
Spin Column[1]	Individually, in inserted in a 2.0 ml Collection Tube[2]
Loading Volume	Maximum 800 µl
RNA Binding Capacity	Maximum 45 µg
Recovery	85 -95% depending on the elution volume
Elution Volume	Generally, eluted with 30 –50 µl of Elution Buffer

1 DonotstoretheColumnpacksundercompletelydriedconditions.ItmaybeaffectedtoRNAbindingcapacity.TheSpinColumns arestableforover2yearunderthesеconditions

2 Additional CollectionTubes(100ea)arealsosuppliedforyourconvenient handling.

Important Points Before Starting

- BufferRWB

  : BufferRWBissuppliedasconcentrate. Beforeusingfor thefirst time, besuretoadd40ml of absoluteethanol (96~100%)toobtainaworkingsolution.

- Centrifugation
  AllcentrifugationstepsarecarriedoutatRT(15-25 ℃)

Note for Sample

IQeasy™ plusBloodRNAExtractionMini Kitcanbeusedforisolatingtotal RNAfromthewhiteblood cellfractionofbloodorbuffycoat.

• Wholeblood

Wholebloodshouldbecollectedinthepresenceof ananticoagulant, suchasEDTA,citrate, heparin canbeused.Foridealresults,bloodsampleshouldbeprocessedwithinafewhoursofcollection.
Enablingbloodsamplevolume:Minimum500µl ~Maximum1.5ml

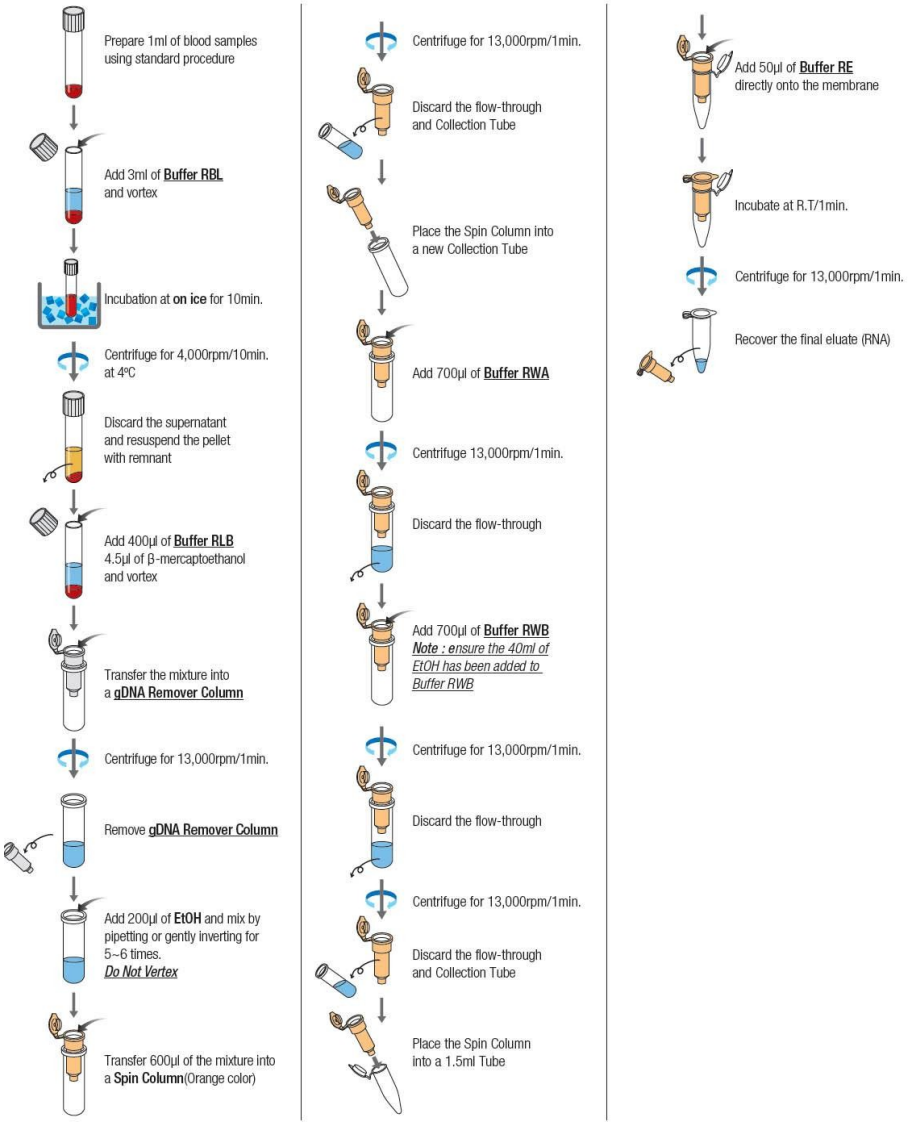
• Buffycoat

Buffycoatisthefractionofacentrifugatedbloodsamplethatcontainmostofthewhitebloodcells.To get thebuffycoat, it isbetter touseafter obtainwholebloodimmediately. Preparebuffycoat by centrifugingwholebloodat3,000rpmfor10minutesatroomtemperature.Aftercentrifugation,onecan distinguishalayerofclearfluid(theplasma),alayerofredfluidcontainingmostoftheredbloodcells, andathinlayerinbetween,thebuffycoat,withmostofthewhitebloodcellsandplatelets.Thebuffy coat isusuallywhitishincolorbut sometimesgreen. Thebuffycoat isusedtoextract RNAfromthe bloodofmammals.
Buffycoatsamplevolume:approximately300µl ofstartamount

Recovery of Purified RNA

Determinationof concentration, yield, andpurityRNAyieldisdeterminedfromtheconcentrationof RNAinelute,measuredbyabsorbanceat260nm.Absorbancereadingsat260nmshouldfall between 0.1and1.0tobeaccurate. Sampledilutionshouldbeadjustedaccordingly: for example, theelute containing4~40ngRNA/µl(A $_{260}$ =0.5~1.0)shouldnotbedilutedwithmorethan4volumesofbuffer. Useelutionbufferorwater(asappropriate)todilutesamplesandtocalibratethespectrophotometer. Measuretheabsorbanceat260and280nm,orscanabsorbancefrom220~320nm(ascanwill show if thereareotherfactorsaffectingabsorbanceat 260nm). BothRNAandRNAaremeasuredwitha spectrophotometer; tomeasureonlyRNA, afluorometer must beused. Purityisdeterminedby calculatingtheratioofabsorbanceat260nmtoabsorbanceat280nm.PureRNAhasanA $_{260}$/A$_{280}$ ratio of 1.8~2.0. RNApurifiedbytheIQeasy ™ plusBloodRNAExtractionMini Kit procedureisfreeof proteinandothercontaminantsthatcaninhibitPCRorotherenzymaticreactions.

IQeasy™ Plus Blood RNA Extraction Mini Kit procedure



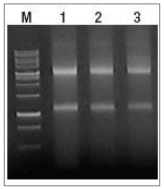
Protocol

Refertothe"VISUALPROTOCOL"

1. **Add1mlofwholebloodtoa15mltube.**
   Note:AmaximumvolumeamountIml ofwholebloodcanbeprocessedonalQeasy ™ plusBloodRNAExtractionMini Kit. Forbloodwithincreasednumberofleukocytes, lessthan1mlmustbeused.
   Note:BloodmustbecollectedinEDTA,heparinandcitrate-coatedtubetopreventclotting.Incaseofusingmorethan300µl ofwholeblood,addBufferRBLatthe3volumeratio(wholeblood:buffer=1:3)asbefore.Makesuretouselargertubefor largervolumeofbloodpreparation. If15mlor50mltubeisused,centrifugeat2,000g.
2. **Add3mlofBufferRBL,andvortex.**
   Note: ThisstepistoremoveRBC. If anyRBCisleft behind, proteinssuchashistonewill hamperexperimentslikePCR. Therefore,itisextremelyimportanttoremoveRBCcompletely.
3. **Incubatefor10min. onice. Centrifugeat4,000rpmfor10min. at4 ℃ toobtainwhitebloodcell pellet. Carefullyremovedsupernatant.**
   Note:IftheRBCarestillvisibleaftercentrifuge, repeat „step2".
4. **Resuspendwhitebloodcell pelletin400µl ofBufferRLBand45µl ofβ-mercaptoethanol (14.2M)to eachsample.Andthenmixbyvortxing.**
   Note:Thisisactual cell lysisstageandisthusimportant toapplyvortexuntil noclumpsareseen.Mixbypipettinguntil no visiblecellclumpsremain.Ifclumpsofcellsarestillvisibleafter5~6timespipettingthesolution.
5. **Apply the lysate to gDNA Remover Spin Columns, and centrifuge for 1min. at 13,000rpm (R.T). After centrifugation, transfer the flow-through into a new 1.5 ml tube.**
   Note: The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
6. **Add200µlofabsoluteethanoltocollectedlysate,andmixitwellbypipettingorgentlyinverting5~6 times.**
   Note:Add0.5volumeofabsoluteethanol tothelystae.
7. **Transfer600µlofthemixturefrom'Step6'intotheSpinColumn(redcolorO-ring)insertedina2.0ml collectiontube.Centrifugeat13,000rpmatR.Tfor1min.,anddiscardflow-throughandcollectiontube altogether.**
   Note:Themaximumvolumeofthecolumnreservoiris800µl.Forsamplevolumesofmorethan800µl,simplyloadandspin again.
8. **Add700µlofBufferRWAtotheSpinColumn,andcentrifugeat13,000rpmfor1min.Discardtheflow-throughandreusethecollectiontube.**
9. **Add700µlBufferRWBtothespincolumn, andcentrifugeat13,000rpmfor1min. todrymembrane. Discardtheflowthroughandcollectiontubealtogether.**
   Note:Ensurethat40µl ofabsoluteethanol (EtOH)hasbeenaddedtoBufferRWB.
10. **PlacetheSpinColumninanew2mlcollectiontube,andcentrifugeat13,000rpmfor1mintodrythe columnmembrane.**
    Note: Itisveryimportant todrythemembraneof thespincolumnsinceresidual ethanol mayinhibitsubsequent reactions. Followingthecentrifugation,removecarefullythespincolumnfromthecollectiontubewithoutcontactingwiththeflowthrough, sincethiswillresultincarryoverofethanol.
11. **Placethespincolumnintoanew1.5ml tube(not supplied), and50µl BufferREdirectlyontothe membrane.Incubatefor1min.atroomtemperatureandthencentrifugefor1min.at13,000rpmtoelute.**
    Note:Elutionwith30µl increasesthefinal RNAconcentration, butreducesoverall RNAyieldconventionally.Alternatively,if youneedlargeramountsofRNA,elutingwith100µl increasesgenerallyoverall RNAyield.

▪ Total RNA preparation from different whole blood amount

IQeasy™ plusBloodRNAExtractionMini Kit for wholebloodisprovidesasimpleandrapidmethodfor the isolationoftotal RNAblood.



[Table1.RNApuritybyperformingratioabsorbancemeasurements.]

Blood volume	500µl	1 ml	1.5 ml
Purity (A_{260}/A_{280})	1.85	1.87	1.8
Yield (µg)	5.2	8.5	10

Fig.1.GelAnalysisoftotalRNAisolatedfromdifferentamountofhumanwholeblood.
Total RNAwaspurifiedfromdifferentamountofhumanwholebloodvolumeusingtheIQeasy ™ plusBloodRNAExtractionMini Kitforwholeblood.Andthentotal RNAwasanalyzedingel electrophoresis.5 µl ofelutedsolutionwasloadedperlaneona 1.0%agarosegel.
LaneM,1KbLadderDNAmarker;**lane1,**1.5mlbloodvolume;**lane2,**1mlbloodvolume;**lane3,**500µl bloodvolume

▪ Real-time RT-PCR amplification of total RNA from whole blood

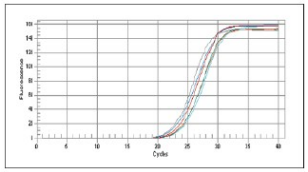
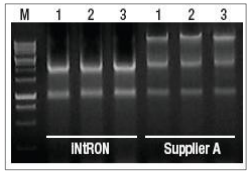


Fig.2.HighreproducibleReal-timeRT-PCR.
Total RNAwaspurifiedfromwholebloodusingtheIQeasy ™ plusBloodRNA ExtractionMini Kit. Total RNAwasanalyzedinreal-timeRT-PCR. Expressionof GAPDHwasthenanalyzedbyReal-timeRT-PCRusing100ngRNAperreaction. Thefigureshows6timesrepetitionplots.

▪ Comparison of total RNA extraction

IQeasy™plusBloodRNAExtractionMini KitshowsanimprovedRNAextractionandgDNAeliminationefficiencyof RNAextractioncomparingwithcompetitor's



[Table2.RNApuritybyperformingratioabsorbancemeasurements.]

Sample	Rabbit blood		Human blood		Buffy coat	
Company	Purity	Yield	Purity	Yield	Purity	Yield
iNtRON	2.0	10.2	1.95	10.12	1.95	9.64
Supplier A	1.9	5.23	1.90	5.01	1.79	5.46

Fig.3.GelAnalysisofTotalRNAisolatedfromwholeblood.
Total RNAwaspurifiedfromwholebloodusingtheIQeasy ™ plusBloodRNAExtractionMini KitandsupplierA.Andthentotal RNAwasanalyzedingel electrophoresis.5µl ofelutedsolutionwasloadedperlaneona1.0%agarosegel.
LaneM,1KbLadderDNAmarker;**lane1,**rabbitblood;**lane2,**humanblood;**lane3,**buffycoat

▪ RNA Q.C data for Microarray

Reliableresultsinreal-timeRT-PCRandmicroarrayanalysisdependonthequalityof theRNAsample. The IQeasy™plusBloodRNAExtractionKitprovideahighqualityofRNAintegritywhichisdeterminedbynucleicacid analysis,whereintactRNAisindicatedbya2:1ratiooftheBandsfor28Sand18SrRNA.

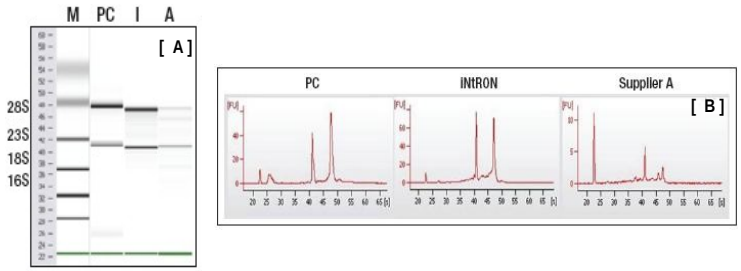


Fig.4.RNAqualitycontroldataformicroarray
Total RNA,isolatedusingtheIQeasy ™ plusBloodRNAExtractionMini KitandSupplierA"sKitwasrunonanAgilent ® 2100 Bioanalyzerandisdisplayedhereasagel electropherogram.
PanelA,Electropherogramgel image;**PanelB,**ElectropherogramSummary
LaneM,RNAMarker;**lanePC,**Positivecontrol;**lanel,**IQeasy ™ plusBloodRNAExtractionMiniKit;**laneA,**SupplierA

▪ Genomic DNA Contamination test

IQeasy™plusBloodRNAExtractionMini Kit integratefast, convenient RNApurificationwitheffectivecontrol of genomicDNAcontamination.leukocytesarelysedinaspecializedbuffer,whichprovidesoptimizedconditionsfor removal ofDNAbyabrief centrifugationthroughagDNARemoverColumn. Total RNAisthenpurifiedusingan IQeasy™SpinColumn.

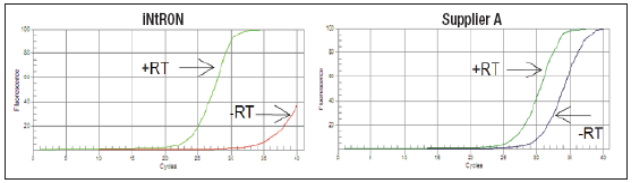


Fig.5.EffectiveeliminationofgenomicDNAcontamination
TotalRNAwaspurifiedfromwholebloodusingtheIQeasy ™ plusBloodRNAExtractionMini Kit.GAPDHtranscriptwasdetected byreal-timeRT-PCR, andCt valueswereobserved. Incontrol reactionswithoutreversetranscriptase(-RT), GAPDHDNAwas notdetectedindicatingtheabsenceofgenomicDNAcontamination.
PanelA,IQeasy ™ plusBloodRNAExtractionMiniKit;**PanelB,**SupplierA"sRNAExtractionMinikit

Troubleshooting Guide

Problem	Problem	Recommendation
Low RNA yield or no RNA	Carryover of erythrocytes	−ExtendRBCLysisincubationoniceto20min.
	Too much starting material	−OverloadingthespincolumnsignificantlyreducesRNAyield. Reducetheamountofstartingmaterial
	RNA still bound to spin column membrane	−RepeatRNAelution,butincubatethespincolumnonthebench top for 10 min with RNase-free water before centrifuging.
	Step were not followed or wrong reagent used	−Checktheprotocol ; 100%EtOHmust beaddedtotheBuffer RWBbeforeuse.
	Inappropriate handling of starting material	−Itisessential toworkquicklyduringsamplepreparation.Perform theRNAextractionprocedurequickly,especiallythefirstfewsteps.
RNA degradation	RNase contamination	−Althoughall buffers havebeentestedandareguaranteed RNase-free,RNasescanbeintroducedduringuse.Becertainnot tointroduceanyRNasesduringtheRNAExtractionprocedureor laterhandling.
	Age of blood	−Bloodsamplestoredfor toolongprior toRNAisolation. or optimal results, bloodsamplesshouldbeprocessedwithinafew hours of collection. mRNAs fromblood cells have different stabilities.mRNAsofregulatorygeneshaveshorterhalf-livesthan mRNAs of housekeeping genes. IQeasy™ plus Blood RNA ExtractionKitcannotbeusedforfrozenbloodsamples.
	Lysis buffer does not contain β-ME	−Ensurethat β-MEhasbeenaddedtothelysisbuffer (Buffer RLB).
Clogged gDNA Remover spin column	Too much starting material	−Reducetheamountofstartingmaterial.Itisessential tousethe correctamountofstartingmaterial
DNA contamination in downstream experiments	Trace amounts of genomic DNA may still remain	−Nocurrentlyavailablepurificationmethodcanguaranteethat RNAiscompletelyfreeofDNA,evenwhenitisnotvisibleonan agarosegel.IQeasyKitswill,however,removethevastmajority ofcellularDNA.gDNARemoverColumnhelpstofurtherreduce genomicDNAcontaminationbuttraceamountsofgenomicDNA maystill remain, dependingontheamount andnatureof the sample.

Related Products

Product	Application	Cat. No.
IQeasy™ plus CTB RNA Extraction Mini Kit	total RNA extraction from Cell, Tissue, Bacteria	17321
IQeasy™ plus Plant RNA Extraction Mini Kit	total RNA extraction from Plant	17491
IQeasy™ plus Viral DNA/RNA Extraction Kit	Viral DNA/RNA extraction from biological samples	17153
ONE-STEP RT-PCR PreMix Kit	One-step RT-PCR premix, solution type	25101
RevoScript™ RT PreMix (Oligo $(dT)_{15}$ Primer)	RT premix (oligo dT primer), aliquoted & dried type	25083/25084
RevoScript™ RT PreMix (Random Primer)	RT premix (random hexamer primer), aliquoted & dried type	25085/25086
Maxime™ RT-PCR PreMix	RT-PCR premix, aliquoted & dried type	25131
Power cDNA Synthesis Kit	cDNA synthesis	25011
SiZer™-100 DNA Marker Solution	100bp DNA size marker	24073
SiZer™-1000 DNA Marker Solution	1kb DNA size marker	24074
RedSafe™ Nucleic Acid Staining Solution	Nucleic acid staning solution, non-toxic and non-carcinogenic	21141
RNase WiPER™	DNA/RNase remover	21131
RealMOD™ Real-time PCR Core Kit	Real-time PCR reagent(for Taqman probe)	25331
RealMOD™ Real-time PCR Master mix Kit	Real-time PCR master mix Solution(for Taqman probe)	25341/25342
RealMOD™ Green Real-time PCR Core Kit	Real-time PCR reagent(for HRM dye)	25332
RealMOD™ Green Real-time PCR Master mix Kit	Real-time PCR master mix Solution(for HRM dye)	25343/25344