Media Sampler Pack

Perfecting purification can require more than one choice of support. Bio-Rad now offers its most popular media in a convenient sampler pack. The media sampler pack allows testing of different techniques at early stages of process development.

Included in the sampler pack are:
- Macro-Prep® High Q, DEAE, High S, and CM ion exchange media
- CHT™ ceramic hydroxyapatite Types I and II
- UNOsphere™ Q and S ion exchange media
- Macro-Prep methyl and t-butyl hydrophobic interaction media

All media are fully supported for regulatory submissions.

Ordering Information

Catalog #	Description
158-0100	Media Sampler Pack

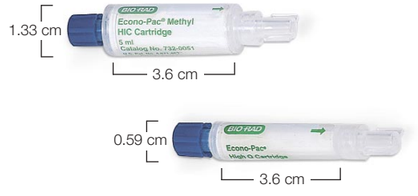
New Econo-Pac® Cartridges

Bio-Rad's convenient and affordable prepacked Econo-Pac cartridges now include the popular UNOsphere™ S, UNOsphere Q, and Macro-Prep® DEAE ion exchange media, and CHT™ ceramic hydroxyapatite Type I. Econo-Pac cartridges simplify chromatography because there are no media to prepare or columns to pack. The cartridges are designed for use with the BioLogic™ systems, a peristaltic pump, any chromatography system, or a syringe for simple step elution.

Cartridge Name	Support	Type	Chemical Form	Protein Capacity	Application
UNOsphere Q	UNOsphere Q	Strong anion exchange	$-N^+(CH_3)_3$	900 mg BSA	Protein, plasmid purification
UNOsphere S	UNOsphere S	Strong cation exchange	$-SO_3^-$	300 mg BSA	Protein purification
DEAE	Macro-Prep DEAE	Weak anion exchange	$-N^+(C_2H_5)_2$	175 mg BSA	Acidic and neutral protein and peptide purification
CHT I	CHT ceramic hydroxyapatite Type I, 20 µm	Ceramic hydroxyapatite	$[Ca_5(PO_4)_3OH]_2$	70 mg BSA	Protein, nucleic acid purification

Ordering Information

Catalog #	Description
Econo-Pac Cartridges	
732-0200	UNOsphere Q Support, 1 x 5 ml
732-0201	UNOsphere Q Support, 5 x 1 ml
732-0202	UNOsphere Q Support, 5 x 5 ml
732-0210	UNOsphere S Support, 1 x 5 ml
732-0211	UNOsphere S Support, 5 x 1 ml
732-0212	UNOsphere S Support, 5 x 5 ml
732-0007	Macro-Prep DEAE Support, 1 x 5 ml
732-0008	Macro-Prep DEAE Support, 5 x 5 ml
732-0009	Macro-Prep DEAE Support, 5 x 1 ml
732-0082	CHT Ceramic Hydroxyapatite Type I Support, 1 x 5 ml
732-0086	CHT Ceramic Hydroxyapatite Type I Support, 5 x 1 ml
732-0084	CHT Ceramic Hydroxyapatite Type I Support, 5 x 5 ml



Updated iCycler iQ® Optical System Software

Obtain the most streamlined data output using the new version 3.1 software for the iCycler iQ optical system. This software update, available to all iCycler iQ customers, puts more analysis tools in the hands of the researcher. The iCycler iQ system software accelerates data analysis and optimizes graphical data output for publication needs.
Features of the version 3.1 update include:
- Optimized curve-fit algorithm
- One-click toggle between logarithmic and linear views of data
- Rigorous autobaselining function

Minimum computer specifications:
Windows NT, Windows 2000, or Windows XP operating system
500 MHz processor
256 MB RAM, 512 MB recommended
1024 x 768 screen resolution with true-color mode (24 or 32 bits)
Microsoft Internet Explorer (v. 5.0 or higher) browser
6 GB hard drive
CD-ROM drive
57.6 kbps serial port
Bidirectional parallel port (EPP)

For your update, contact your local sales representative or call Technical Support at 1-800-4BIORAD.
For more information on Bio-Rad's entire amplification product line, visit us on the Web at **www.bio-rad.com/amplification/**

Ordering Information

Catalog #	Description
170-8754	iCycler iQ Optical System Software Version 3.1

MyCycler™ Sample Loading Tray

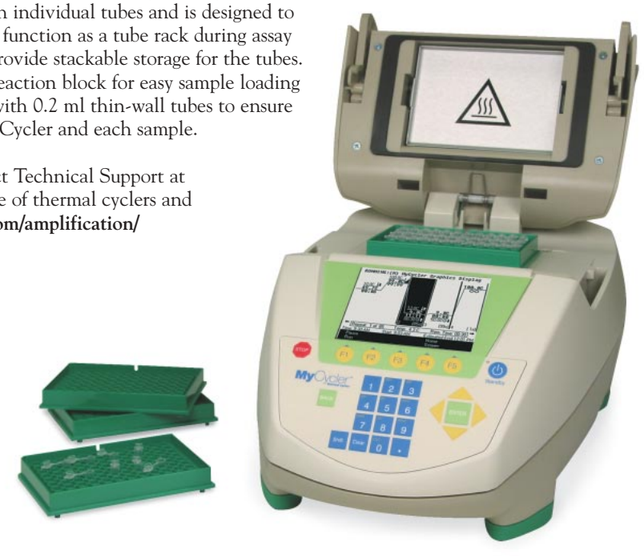
The MyCycler sample loading tray is the newest accessory for the MyCycler personal thermal cycler. The 96-well tray is compatible with individual tubes and is designed to simplify working with thin-wall PCR tubes. It can function as a tube rack during assay setup, support tubes during thermal cycling, and provide stackable storage for the tubes.

The tray is placed directly over the MyCycler reaction block for easy sample loading and removal, and is highly recommended for use with 0.2 ml thin-wall tubes to ensure uniform contact between the heated lid of the MyCycler and each sample.

To request a MyCycler sample loading tray, contact Technical Support at 1-800-4BIORAD. To view Bio-Rad's complete line of thermal cyclers and accessories, visit us on the Web at **www.bio-rad.com/amplification/**

Ordering Information

Catalog #	Description
170-9709	MyCycler Sample Loading Tray

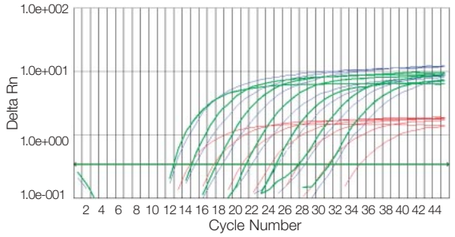


iTaq™ SYBR® Green Supermix With ROX

iTaq SYBR Green supermix with ROX is formulated to easily achieve optimal results in real-time quantitative PCR assays on all ROX-dependent instrument platforms. This formula delivers high performance with cDNA as well as genomic and plasmid DNA templates over a broad dynamic range, achieving sensitive and specific amplification over at least 7 orders of magnitude. The supermix is preblended with hot-start iTaq DNA polymerase, optimized buffer, nucleotides including dUTP for optional UNG-mediated treatment of carryover contamination, SYBR Green I dye, and ROX passive reference dye. Its convenient one-tube formulation will enable you to obtain specific and sensitive amplification every time.

- Proprietary low-foam formulation enables smoother pipetting
- Validated for use on all ROX-dependent optical thermal cycler platforms
- Conveniently preblended with ROX and dUTP

For more information, request bulletin 3065.



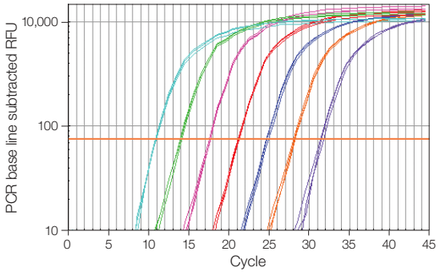
Bio-Rad's supermix gives superior results on the ABI PRISM 7000 sequence detection system. iTaq SYBR Green supermix with ROX (green) and other ROX-containing supermixes (red and blue) were used to amplify 10-fold serial dilutions (10^7 to 10 copies) of the GAPDH gene in a plasmid template. The Bio-Rad supermix consistently generated earlier C_T values than the competitors' mixes, and produced high ΔR_n values.

iScript™ One-Step Quantitative RT-PCR Kits

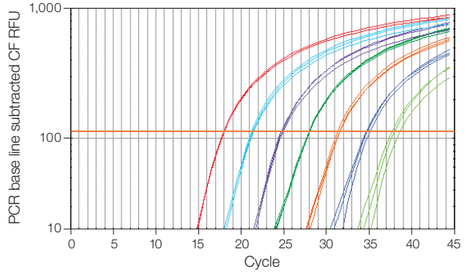
The iScript one-step RT-PCR kits (with or without SYBR Green) are highly sensitive solutions for real-time quantitative RT-PCR on a broad range of optical thermal cyclers. cDNA synthesis and PCR amplification are conveniently carried out in the same tube, using the powerful iScript reverse transcriptase and iTaq™ hot-start DNA polymerase combination. These kits are optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity without compromising fluorescent signal. The proprietary reaction buffers have been formulated for optimal activity of both iScript reverse transcriptase and iTaq DNA polymerase, while minimizing the potential for formation of primer-dimers and other nonspecific PCR artifacts. The kits are easy to use and are perfectly suited for a broad range of real-time PCR applications.

- Highly specific amplification over a broad dynamic range
- Extremely sensitive detection down to 100 fg of input RNA
- Convenient one-tube setup minimizes handling and contamination risk

For more information, request bulletin 3066.



The iScript one-step RT-PCR kit with SYBR Green provides high sensitivity across a broad range of concentrations. One-step RT-PCR reactions were performed in quadruplicate, along with no-template controls, using GAPDH primers and 100 ng to 100 fg of total HeLa RNA. Reactions were carried out on the iCycler iQ® real-time system. Standard curve had r = 1.000, slope = –3.466, efficiency = 95%.



Bio-Rad's iScript one-step RT-PCR kit for probes gives accurate and streamlined results with any detection chemistry. RNA (100 ng to 100 fg) isolated from HeLa cells using the Aurum total RNA kit was reverse transcribed and amplified using primers to β-actin and a FAM-labeled detection probe. The reactions were carried out on the MyiQ™ real-time system. Standard curve had r = 1.000, slope = –3.360, efficiency = 98.4%.

Ordering Information

Catalog #	Description
iTaq SYBR Green Supermix With ROX	
170-8850	iTaq SYBR Green Supermix With ROX, 200 x 50 µl reactions
170-8851	iTaq SYBR Green Supermix With ROX, 500 x 50 µl reactions
170-8852	iTaq SYBR Green Supermix With ROX, 1,000 x 50 µl reactions

Ordering Information

Catalog #	Description
iScript One-Step Quantitative RT-PCR Kits	
170-8892	iScript™ One-Step RT-PCR Kit With SYBR® Green, 50 reactions
170-8893	iScript One-Step RT-PCR Kit With SYBR Green, 200 reactions
170-8894	iScript One-Step RT-PCR Kit for Probes, 50 reactions
170-8895	iScript One-Step RT-PCR Kit for Probes, 200 reactions
Other PCR Reagents	
170-8890	iScript cDNA Synthesis Kit, 25 x 20 µl reactions
170-8891	iScript cDNA Synthesis Kit, 100 x 20 µl reactions
170-8860	iQ™ Supermix, 100 x 50 µl reactions
170-8862	iQ Supermix, 500 x 50 µl reactions
170-8864	iQ Supermix, 1,000 x 50 µl reactions
170-8880	iQ SYBR Green Supermix, 100 x 50 µl reactions
170-8882	iQ SYBR Green Supermix, 500 x 50 µl reactions
170-8884	iQ SYBR Green Supermix, 1,000 x 50 µl reactions
170-8870	iTaq DNA Polymerase, 250 U
170-8872	$MgCl_2$ Solution, 50 mM, 1.25 ml
170-8874	dNTP Mix, 200 µl of 10 mM of each dNTP

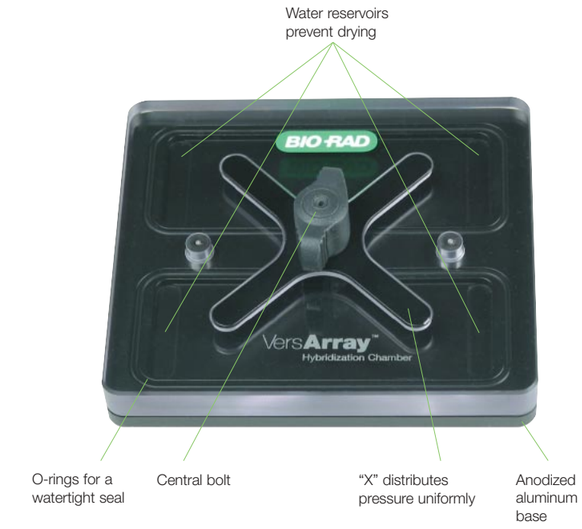
VersArray™ Hybridization Chamber

The Ultimate Tool for Microarray Hybridizations

The VersArray hybridization chamber is designed to increase the effectiveness of manual (coverslip) hybridizations. It allows the quick and easy loading of one or two slides, permits superior thermal transfer, and is watertight to allow hybridization in a water bath. The chamber is easily opened and closed using the single attachment bolt, and a unique patent-pending pressure mechanism ensures uniform sealing.

Key Benefits

- Simultaneous processing of two microarray slides
- Watertight — allows water bath incubations
- One attachment bolt for easy and convenient opening and closing
- Water reservoirs (2 per slide) prevent slides from drying out
- Aluminum composition promotes uniform heat transfer



Ordering Information

Catalog #	Description
169-0500	VersArray Hybridization Chamber

film-based detection method, which must be developed by trial and error, and it doesn't use costly materials such as X-ray film and darkroom chemicals.

Colorimetric samples can be easily recorded and analyzed with a densitometer such as the GS-800™ calibrated densitometer. The densitometer provides a digital record of the blot, excellent resolution, reproducible results, and accurate quantitation. The GS-800 also uses red-, green-, and blue-color CCD technology to greatly improve the detection of a wide range of colorimetric detection reagents.

Colorimetric detection is typically considered a medium-sensitivity method compared to radioisotopic or chemiluminescent detection. However, Bio-Rad has colorimetric systems that offer very high sensitivity matching that of chemiluminescence. These include the Immun-Blot® amplified AP assay kit, which uses a biotinylated secondary antibody and a streptavidin-biotinylated AP complex to amplify the signal, and the HRP-based amplified Opti-4CN™ kit.

Bio-Rad offers many products for blotting and blot detection. For more information, request Bio-Rad's western blotting products folder (bulletin 2033) or visit us on the Web at **discover.bio-rad.com**

Ordering Information

Catalog #	Description
Immun-Star HRP Chemiluminescent Detection Kits	
170-5040	Immun-Star HRP Substrate, 500 ml
170-5041	Immun-Star HRP Substrate, 100 ml
170-5042	Goat Anti-Rabbit-HRP Detection Reagents
170-5043	Goat Anti-Mouse-HRP Detection Reagents
170-5044	Goat Anti-Mouse-HRP Detection Kit
170-5045	Goat Anti-Rabbit-HRP Detection Kit
Immun-Star AP Chemiluminescent Detection Kits	
170-5018	Immun-Star AP Substrate
170-5010	Goat Anti-Mouse-AP Detection Kit
170-5011	Goat Anti-Rabbit-AP Detection Kit
170-5012	AP Substrate Pack
170-5013	Goat Anti-Mouse-AP Intro Kit
170-5014	Goat Anti-Rabbit-AP Intro Kit
170-5015	Blotting Reagents Pack
Immun-Blot AP and HRP Colorimetric Assay Kits	
170-6460	Goat Anti-Rabbit IgG (H + L)-AP Assay Kit
170-6461	Goat Anti-Mouse IgG (H + L)-AP Assay Kit
170-6462	Goat Anti-Human IgG (H + L)-AP Assay Kit
170-6412	Immun-Blot Amplified AP Assay Kit
170-6463	Goat Anti-Rabbit IgG (H + L)-HRP Assay Kit
170-6464	Goat Anti-Mouse IgG (H + L)-HRP Assay Kit
170-6465	Goat Anti-Human IgG (H + L)-HRP Assay Kit
170-8238	Amplified Opti-4CN Substrate Kit
170-8235	Opti-4CN Substrate Kit
Premixed Substrate Reagents	
170-6431	HRP Conjugate Substrate Kit
170-6432	AP Conjugate Substrate Kit
Blot Transfer and Processing Buffers	
161-0778	10x Tris/CAPS, 1 L
161-0774	20x SSC, 1 L
161-0780	10x PBS, 1 L
161-6435	10x TBS, 1 L
161-0781	10% Tween 20, 1 L
170-6531	Tween 20, EIA grade, 100 ml
161-0783	1x Phosphate Buffered Saline With 1% Casein, 1 L
161-0782	1x Tris Buffered Saline With 1% Casein, 1 L
Blotting Membranes	
162-0232	0.2 µm Nitrocellulose/Filter Paper Sandwiches, 8.5 x 13.5 cm, 20 pack
162-0233	0.2 µm Nitrocellulose/Filter Paper Sandwiches, 8.5 x 13.5 cm, 50 pack
162-0234	0.45 µm Nitrocellulose/Filter Paper Sandwiches, 8.5 x 13.5 cm, 20 pack
162-0235	0.45 µm Nitrocellulose/Filter Paper Sandwiches, 8.5 x 13.5 cm, 50 pack
162-0238	Immun-Blot PVDF/Filter Paper Sandwiches, 8.5 x 13.5 cm, 20 pack
162-0239	Immun-Blot PVDF/Filter Paper Sandwiches, 8.5 x 13.5 cm, 50 pack

Catalog #	Description
Blotting Membranes (cont.)	
162-0236	Sequi-Blot™ PVDF/Filter Paper Sandwiches, 8.5 x 13.5 cm, 20 pack
162-0237	Sequi-Blot PVDF/Filter Paper Sandwiches, 8.5 x 13.5 cm, 50 pack
162-0212	0.2 µm Nitrocellulose/Filter Paper Sandwiches, 7 x 8.5 cm, 20 pack
162-0213	0.2 µm Nitrocellulose/Filter Paper Sandwiches, 7 x 8.5 cm, 50 pack
162-0214	0.45 µm Nitrocellulose/Filter Paper Sandwiches, 7 x 8.5 cm, 20 pack
162-0215	0.45 µm Nitrocellulose/Filter Paper Sandwiches, 7 x 8.5 cm, 50 pack
162-0218	Immun-Blot PVDF/Filter Paper Sandwiches, 7 x 8.5 cm, 20 pack
162-0219	Immun-Blot PVDF/Filter Paper Sandwiches, 7 x 8.5 cm, 50 pack
162-0216	Sequi-Blot PVDF/Filter Paper Sandwiches, 7 x 8.5 cm, 20 pack
162-0217	Sequi-Blot PVDF/Filter Paper Sandwiches, 7 x 8.5 cm, 50 pack
162-0177	Immun-Blot PVDF Membrane, 26 cm x 3.3 m
162-0184	Sequi-Blot PVDF Membrane, 24 cm x 3.3 m
162-0115	Nitrocellulose Membrane, 0.45 µm, 30 cm x 3.5 m
162-0112	Nitrocellulose Membrane, 0.2 µm, 30 cm x 3.5 m
162-0094	Supported Nitrocellulose Membrane, 0.45 µm, 30 cm x 3 m
162-0095	Supported Nitrocellulose Membrane, 0.2 µm, 30 cm x 3 m
Blotting Apparatus	
170-3930	Mini Trans-Blot® Electrophoretic Transfer Cell
170-4070	Criterion™ Blotter With Plate Electrodes
170-4071	Criterion Blotter With Wire Electrodes
170-3939	Trans-Blot® Cell With Plate Electrodes
170-3940	Trans-Blot SD Semi-Dry System
Power Supplies	
164-5052	PowerPac™ HC Power Supply
164-5050	PowerPac™ Basic Power Supply
Imaging Equipment	
170-7850	Molecular Imager FX Pro Plus System, PC
170-7851	Molecular Imager FX Pro Plus System, Mac
170-8030	VersaDoc Model 3000 Imaging System, PC
170-3031	VersaDoc Model 3000 Imaging System, Mac
170-8140	VersaDoc Model 4000 Imaging System, PC
170-8141	VersaDoc Model 4000 Imaging System, Mac
170-7980	GS-800 Calibrated Densitometer, PC
170-7981	GS-800 Calibrated Densitometer, Mac
170-8070	ChemiDoc XRS System, PC
170-8071	ChemiDoc XRS System, Mac
Background Remover	
170-5020	DeExpose™ Background Remover, 10x, 250 ml

Standard Curves as Troubleshooting Tools for Real-Time RT-PCR Assays

Real-time PCR is a powerful technique with a broad range of applications. The most common application is evaluating mRNA levels by reverse transcription PCR (RT-PCR). This is also one of the more challenging applications, as it involves two distinct enzymatic reactions — reverse transcription and PCR — and the use of internal controls that substitute for the more ideal normalization to cell number.

This article focuses on standard curves generated from different templates available to the researcher. Using different templates to evaluate real-time PCR assays can provide valuable information, even if your experimental design does not require that standard curves be run.

Standard curves derived from serial dilutions of samples provide a useful tool to evaluate the consistency of these enzymatic reactions. These experiments test the response of your reagent system to different starting quantities. Similar to a mathematical formula, the assay should return predictable and consistent results based on the inputs. The equation for the standard curve is in fact a mathematical function describing the assay in question.

Figure 1A shows an example of a standard curve run with a 100-fold dilution series, from 10^8 to 10^2 copies, of plasmid DNA. The fact that the curve is linear demonstrates that the assay has responded consistently to all concentrations of template tested. The equation for the curve is the mathematical function, and the red line is a graphical representation of that function. The fact that all of the standards fall on the curve indicates that the assay is returning the expected threshold cycle (C_T) values for the tested input quantities. r^2 is the correlation coefficient squared and is a measure of how closely the calculated C_T values fit the expected values. r^2 is a positive number, and the closer to 1.000, the better the fit.

Even if you are using cDNA, this type of experiment may be important to run because a plasmid DNA template has none of the confounding factors that might be present in samples isolated from an experimental system, reverse transcribed, and stored over a period of time.

Running the experiment on a dilution series of cDNA provides you with additional information. Figure 1B is an example of one such assay. This 10-fold dilution series of cDNA represents 100 ng down to 10 pg of RNA. Note that the 100 ng standard does not fall on the standard curve. The assay is not returning the expected C_T value for that concentration of cDNA. Additionally, the efficiency of the assay derived from the slope of the standard curve is 121% — 21% higher than the theoretical limit. Both of these factors indicate a problem in the experimental system.

Excluding the problematic 100 ng standard from the data set allows the other four standards to fall on a straight line, and the derived efficiency is now a more acceptable 101% (Figure 1C).

Results of this kind, in which one or more standards do not fall on a straight line, are not uncommon and are usually the result of some kind of inhibitor present in the cDNA sample. The inhibitor is diluted out at the lower concentrations, so it does not affect the kinetics of the experiment at these concentrations. The fact that this results in >100% efficiency may be counterintuitive, but remember that the efficiency value is derived from the slope of the standard curve. The inhibitor is causing the high-concentration standard to have a higher C_T value than it should. This makes the slope shallow, thus resulting in a derived efficiency over 100%. It is often necessary to examine the traces of the real-time amplifications (not shown) and assess the slopes to determine if the individual efficiencies are approximately equivalent. All traces should be

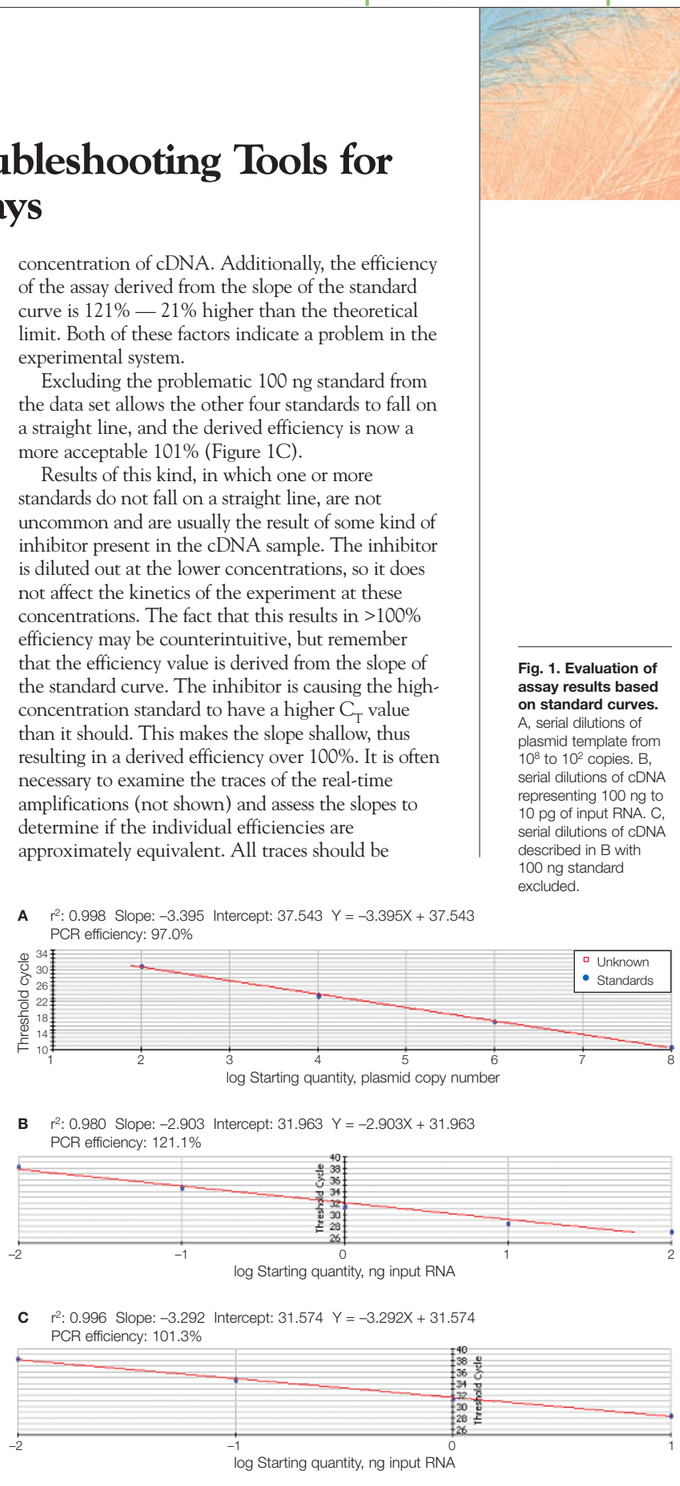


Fig. 1. Evaluation of assay results based on standard curves. A, serial dilutions of plasmid template from 10^8 to 10^2 copies. B, serial dilutions of cDNA representing 100 ng to 10 pg of input RNA. C, serial dilutions of cDNA described in B with 100 ng standard excluded.

parallel when viewed with the y-axis on the log scale.

Possible sources of inhibition are organic solvents carried over from RNA isolation, inhibitory proteins not removed during isolation, and ethanol left from the precipitation or wash steps. It is also important to heat-inactivate the reverse transcriptase, as it too will inhibit your PCR reaction.

How Does This Apply to Your Research?

An important and often overlooked consideration is evaluating the PCR reaction without the confounding factors presented by the biological sample. In addition to being heterogeneous, cDNA samples are obtained from sources that may contain inhibitors. The isolation process itself may also introduce inhibiting compounds. In some cases the effect of these impurities is not as clear as in the example given. Results may not be reproducible at any concentration. There may be no three consecutive standards that actually show a linear relationship. When results like these are obtained, the next logical step is to use a high-purity plasmid or gel-isolated PCR product to evaluate the assay.

Comparing the results of serial dilutions of clean, homogeneous samples like purified plasmid to representative cDNA samples can yield valuable information about your assay. The plasmid experiment reflects the true kinetics of an assay without the confounding factors that can be introduced with cDNA. The serial dilutions of representative cDNA demonstrate the performance of the assay in the real-world experiment, namely samples from the biological system being studied. Running only the cDNA experiment may lead you to redesign a perfectly acceptable assay when sample preparation is the only problem. Performing the plasmid experiment tests the assay in ideal conditions.

There is one more serial dilution that should be performed to assess your experimental conditions. So far the discussion has focused on the PCR reaction's response to different sample sources. The other enzymatic reaction in RT-PCR-based expression analysis is the reverse transcription (RT) reaction. This reaction can be conceptualized as a simple enzymatic reaction involving an enzyme, reverse transcriptase, and mRNA as the substrate, though it does not fit the classical definition of a substrate. The cDNA is clearly the product of interest. This enzymatic reaction can be saturated and does have a limit of detection. Therefore, you should test these parameters of the RT reagent system with varying concentrations of RNA to verify that the system is reverse transcribing RNA to cDNA reproducibly and predictably. A dilution series of RNA is prepared, and then each of these dilutions is reverse transcribed (Figure 2A). The proportion of cDNA transcript produced from each RNA dilution should be the same.

The resulting cDNA samples are then analyzed using a real-time PCR assay. The resulting standard curve should be linear and have the same slope (efficiency) as a curve produced by a dilution series of cDNA (Figure 2B). Figure 2C demonstrates the results of such an experiment performed to test Bio-Rad's iScript™ cDNA synthesis kit.

Whether you are evaluating the PCR reaction or the RT reaction, the information obtained from standard curves can be used to make decisions about experimental design. Only concentrations that produce linear standard curves should be used, and aberrant slopes or efficiencies should be investigated. The RT reaction in particular is susceptible to saturation and sensitivity issues. The PCR reaction, though usually not susceptible to saturation, may be inhibited by contaminants. Generating standard curves allows you to evaluate whether these factors are likely to become problematic, and at what concentration. You can then proceed with an appropriate strategy with the experimental samples of your system of interest.

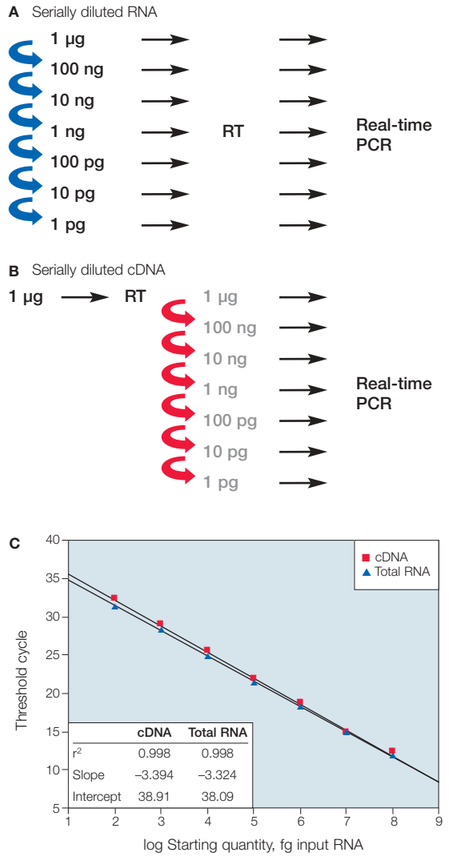


Fig. 2. Experimental evaluation of reverse transcription (RT) reactions. A, serial dilutions of RNA were prepared and reverse transcribed, followed by quantitation of the resulting cDNA by real-time PCR. B, after reverse transcription of 1 µg RNA, the resulting cDNA was serially diluted and quantitated by real-time PCR. Ghosted values indicate theoretically equivalent amounts of starting RNA. C, the resulting standard curves were essentially the same.

Prevention of Vertical Streaking

Tom Berkelman, Mary Grace Brubacher, and Haiyin Chang, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 USA

In the last issue of BioRadiations (111), we discussed the various causes of horizontal streaking on 2-D gels, and its prevention. Horizontal streaking is the result of problems that occur during the first-dimension separation (generally performed in IPG strips). This article focuses on vertical streaking, which results from problems related to second-dimension separation. Vertical streaking should not be confused with vertical gaps or blank regions in the 2-D pattern (see final paragraph). Examples of vertical streaking are presented in Figure 1.

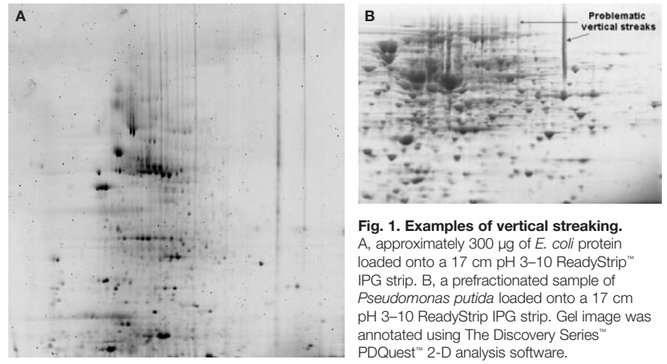


Fig. 1. Examples of vertical streaking. A, approximately 300 µg of *E. coli* protein loaded onto a 17 cm pH 3–10 ReadyStrip™ IPG strip. B, a prefractionated sample of *Pseudomonas putida* loaded onto a 17 cm pH 3–10 ReadyStrip IPG strip. Gel image was annotated using The Discovery Series™ PDQuest™ 2-D analysis software.

Poor Protein Solubility

Following the first-dimension separation, all of the proteins have moved to their isoelectric points and carry no net charge. The ionic strength of the medium is also very low, because small ions have all migrated out of the first-dimension strip. Proteins generally have minimal solubility under these conditions and are often precipitated within the gel matrix despite the presence of detergents, chaotropic agents, and other solubilizing additives. The major purpose of the equilibration step following the first dimension is to coat the proteins with SDS, giving them a negative charge and making them soluble again so that they may enter the second-dimension gel.

High Protein Load

When a large quantity of protein is loaded, or when the sample contains proteins of particularly high abundance, resolubilization during equilibration may be incomplete, resulting in vertical streaking and tailing of the most intense protein spots. This can be prevented by limiting the amount of protein loaded onto the first-dimension strip. It is often possible to compensate for a lower protein load by using a more sensitive staining technique (for example, silver stain, catalog #161-0449, or SYPRO Ruby protein gel stain, catalog #170-3125, instead of Coomassie Blue stain). In some cases, vertical streaking of abundant proteins can be prevented by prolonging the equilibration time (see "Ineffective Equilibration" below).

Overfocusing

Isoelectric precipitation increases with focusing time, so vertical streaking may be minimized by ensuring that first-dimension IEF is not conducted for any longer than necessary.

Ineffective Equilibration

Equilibration should be carried out in a manner that ensures good penetration of SDS and therefore protein solubilization. The equilibration tray should be shaken or rocked to ensure continual movement of solution. Equilibration may be prolonged to as long as 45 minutes if necessary. This may result in the loss of some small polypeptides (<15 kD), but loss of larger proteins is generally insignificant even with prolonged equilibration. SDS should be present at a concentration of at least 2% (w/v), particularly if high (>2%) concentrations of detergent were used in the first dimension. Other components of the equilibration solution are important as well. The solution should be buffered, particularly if an alkylation step is employed, because iodoacetamide treatment generates acid. The optimal pH for equilibration is 8.8. Glycerol, which should be present at a concentration of at least 20% (v/v), is a particularly important component of the equilibration solution, and its omission will result in vertical streaking.

Protein Oxidation

Oxidative crosslinking or protein refolding should be prevented during all steps of the 2-D process; protein oxidation during the second dimension separation can result in vertical streaking. Treatment with the ReadyPrep™ reduction-alkylation kit (catalog #163-2090) prior to first-dimension focusing can block cysteine sulfhydryl groups and prevent their reoxidation. If the sample is not alkylated, alkylation should be performed in a second equilibration step with 2.5% iodoacetamide. If alkylation is not