

QuickPick™ DNA Fragment

71001D • QuickPick™ DNA Fragment DEMO kit, 24 preps
71011 • QuickPick™ DNA Fragment kit, 3 x 96 preps



For

- DNA Purification from Agarose Gels
- DNA Purification from PCR
- DNA Reaction Clean-Up

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INTRODUCTION

These are the instructions for use for the QuickPick™ DNA Fragment kit. Please read the entire instructions carefully before starting to work with the reagents. The QuickPick DNA Fragment reagents are intended for use with the PickPen® magnetic tools supplied by BN Products & Services. Also refer to the PickPen® instructions for use. PickPen® 1-M is recommended when working in microtube format and PickPen® 8-M when working in standard or deep-well microplate format typically with higher throughput.

The QuickPick™ DNA Fragment kit provides a fast and simple means of isolating DNA fragments from gel or from liquid solutions. The kit and Bio-Nobile's PickO™ gel excisers are suitable for use with standard and low-melting agarose gels in TAE or TBE buffer. The technique does not require organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. DNA purified using the QuickPick™ DNA Fragment kit is suitable for DNA sizes from 60 bp to 50 kbp. Purified DNA fragments can be used for downstream applications such as automated fluorescent DNA sequencing, manual DNA sequencing, PCR, in vitro transcription/translation, restriction mapping, restriction enzyme digestion, cloning and labelling. DNA fragments can be eluted both into Elution Buffer or sterile water. The incubation of elution mixture at elevated temperatures (e.g. 50 °C) is preferred for maximizing the yields.

SPECIFICATIONS

Table 1. Specifications of the QuickPick™ DNA fragment kit

Sample materials: DNA fragment size in agarose gel Gel type Buffer type Max weight of gel piece one prep	60 bp – 50 kbp Any type of agarose gel TAE or TBE buffer 100 ± 20 mg
Recovery %: 60 bp – 50 kbp	> 80 %
Maximal binding capacity / prep:	10 µg
Typical purity*:	≥ 1.8
Elution volume:	5 – 100 µl
Total protocol time:	< 15 min

*Ratio of absorbance at 260/280 nm corrected with absorbance at 320 nm

KIT CONTENTS

	71001D	71011
DNA Fragment Magnetic Particles	250 µl	2 x 1.6 ml
DNA Fragment Gel melting/DNA binding Buffer	7.5 ml	95 ml
DNA Fragment Wash Buffer 1	28 ml*	2 x 170 ml*
DNA Fragment Wash Buffer 2	13 ml	2 x 80 ml
DNA Fragment Elution Buffer	1.8 ml	28 ml
8-Pack PickPen [®] Tips	3 packs	not included
PickO [™] gel exciser	24 pcs	not included

*Delivered without ethanol. Add ethanol (96-100%) to Wash Buffer 1 (23 ml for 71011D and 140 ml to both bottles for 71001) before use. The volume of ethanol to be added is also shown on the bottle labels. The volumes indicated here are total volumes after addition of ethanol.

The components for kit 71011 can also be bought separately:

71100 DNA Fragment Magnetic Particles	1.6 ml
71300 DNA Fragment Gel melting/DNA binding Buffer	95 ml
71510 DNA Fragment Wash Buffer 1	170 ml
71520 DNA Fragment Wash Buffer 2	80 ml
71600 DNA Fragment Elution Buffer	28 ml

The QuickPick[™] DNA Fragment kits should be stored at room temperature.

PRINCIPLE OF THE METHOD

DNA fragments in the agarose gel are released using Gel melting/DNA binding Buffer which contains chaotropic salts. Released DNA is bound to silica-based magnetic particles (MP) in the presence of Gel melting/DNA binding Buffer (Vogelstein and Gillespie, 1979). MPs with the bound DNA are captured with PickPen[®] and contaminants are removed by washing twice with Wash Buffer 1. A unique and proprietary DipWash[™] method is used in the next step to eliminate ethanol traces. During the DipWash[™] the MPs are not released from the PickPen[®] tool, instead the MPs are dipped shortly into Wash Buffer 2. Finally, DNA is eluted from the MPs with Elution buffer, TE buffer or sterile water. The procedure starting from prepared sample and ending with purified DNA lasts less than 15 minutes.

Note: The use of silica particles for the purification of DNA from complex biological samples is covered by U.S patent 5,234,809 (Boom et al., 1993). The QuickPick[™] DNA Fragment kit is intended for use to the purification of DNA from pre-purified DNA-containing samples.

Precautions should be taken during purification of longer DNA fragments (10 kb) to avoid shearing. Mix preferably by finger tapping or inverting the tubes.

Proteins and RNA do not bind to silica MPs and are eliminated during washes. Neither do silica-based MPs bind oligonucleotides (< 50 bp), allowing the kit to be applied for clean-up of low molecular weight oligonucleotides or nucleotides from DNA.

In summary the QuickPick™ DNA Fragment kit can be applied for the following purposes:

- purify DNA from any type of agarose
- concentrate DNA (for changing buffer, desalting)
- remove proteins (after restriction enzyme treatment; dephosphorylation with BAP or CIAP)
- remove unincorporated nucleotides, primer, primer-dimers and enzymes from a labeling reaction or PCR
- remove an excess of linkers after ligation
- remove residual phenol, chloroform, and ethidium bromide

Vogelstein, B. and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. *Proc.Natl. Acad. Sci. USA*, 76, 615-619.

Boom, W.R., Adriaanse, H.M.A., Kievets, T. and Lens, P.F. (1993) Process for isolating nucleic acid. US Patent 5,234,809.

REAGENT SCALING FOR MANUAL PURIFICATIONS

For manual protocols the reagent amounts are scaled according to sample amounts.

Table 2. The reagent volumes needed in relation to gel weight and liquid sample volumes. One DNA Fragment kit preparation is defined for purification of one PickO™ excised gel piece (approximately 100 mg).

Weight of gel	No. of gel pieces with PickO	Gel melting/Binding Buffer	Magnetic Particles	Wash Buffer 1	Wash Buffer 2	Elution Buffer
Up to 100 mg	1	300 µl	10 µl	2 x 500 µl	500 µl	5 -100 µl
Up to 200 mg	2	600 µl	20 µl	2 x 600 µl	600 µl	5 -100 µl
Up to 300 mg	3	900 µl	30 µl	2 x 700 µl	700 µl	5 -100 µl
Sample amount		Gel melting/Binding Buffer	Magnetic Particles	Wash Buffer 1	Wash Buffer 2	Elution Buffer
Up to 50 µl	-	150 µl	5 µl	2 x 200 µl	200 µl	5 -100 µl
Up to 100 µl	-	300 µl	10 µl	2 x 300 µl	300 µl	5 -100 µl
Up to 200 µl	-	600 µl	20 µl	2 x 400 µl	400 µl	5 -100 µl

SAMPLE PREPARATION

Excision of DNA fragments from agarose gels

The excision of a gel piece using conventional methods i.e. scalpels and razor blades has been a tedious step in molecular biology resulting readily in extended UV exposure increasing the risk of damage to DNA samples. Moreover, the excised gel pieces usually are non-uniform leading to need for weighting of the individual gel slices for determination of the correct reagent use. To increase the efficiency and safety of the process BN Products & Services recommends the use of PickO™ gel exciser method for DNA sample gel-extraction (Fig 1). PickO™ gel exciser method is based on the use of disposable, elastic silicone rubber tube, PickO™, for the picking of the gel piece containing the desired DNA fragment. The method is a one-hand process with high efficiency. Combined with the QuickPick™ DNA fragment kit it will give an easy and fast process for DNA purification from gels.

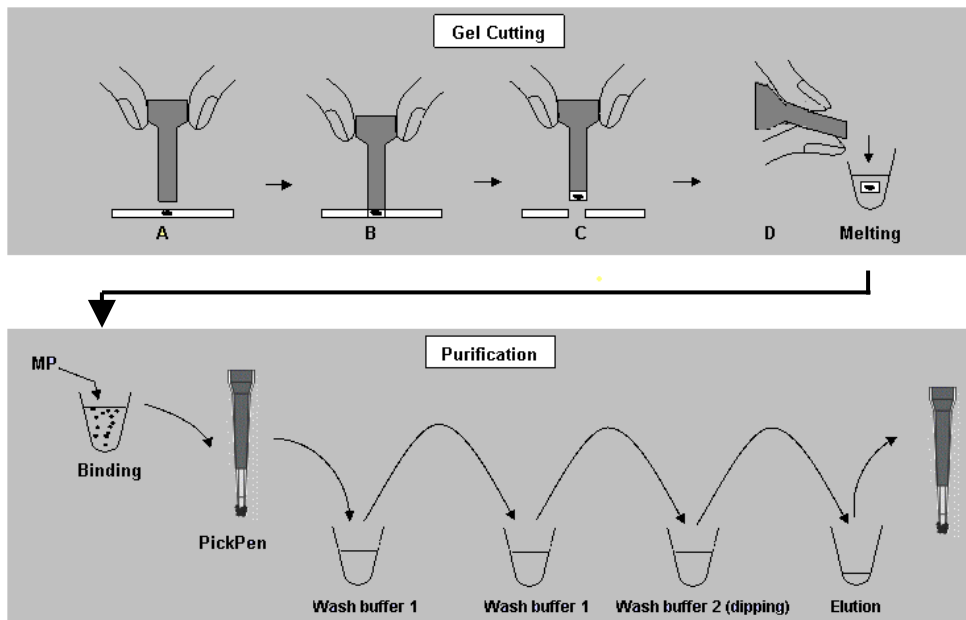


Figure 1. The excision of gel piece using PickO™ gel exciser and the following purification steps with QuickPick™ DNA fragment kit.

DNA fragment excision with PickO™ gel exciser

Excise the DNA fragment of interest from the agarose gel with the PickO™ according to the following instructions. Place the PickO™ above the fragment by looking through the PickO™. Press the PickO™ through the gel. If the gel is hard, press and rotate the PickO™ at the same time. Pick up the gel piece by rotating and bending the PickO™. Transfer the gel piece to the microtube by pressing the upper part of the PickO™. Continue purification step with adding 3 volumes of DNA Fragment Gel melting/DNA binding Buffer to 1 volume of gel (100 mg ~ 100 µl).

The weight of the excised gel piece is dependent on the thickness of the gel and the concentration of the agarose used. Therefore it is recommended that the PickO™ user practice the use of PickO™ and weighs the commonly used agarose gel pieces before actual sample excising. By doing this the user can determine the amount of chaotrope needed for each gel piece in every gel purification.

In routine use the preheated Gel melting/DNA binding Buffer can be added into a container before adding the gel piece which speeds up the melting step.

DNA fragment excision with scalpel / razor blade

Excise the desired DNA fragment by using a clean, sterile razor blade or scalpel. Cut the gel as close to the band as possible and transfer it to a clean, pre-weighted microtube. Use a 1.5 ml tube or plate if the gel piece weighs less than 250 mg and a 2 ml tube or plate for up to 400 mg. If the gel piece weighs more than 400 mg, make sure that the PickPen® device fits into the used container. The final suspension volume will be 4 times the weight of the gel slice. Weigh the gel slice and continue purification step by adding 3 volumes of DNA Fragment Gel melting/DNA binding Buffer to 1 volume of gel (100 mg ~ 100 µl).

Note: The DNA band should be visualized with a medium or long wavelength (e.g. 300-315 nm) UV light, and should be excised quickly to minimize exposure of the DNA to UV light (< 1 min). If the excised gel piece containing the DNA fragment is not purified in the same day it can be stored at -20°C.

Caution: Ultraviolet radiation and EtBr in a gel are dangerous. Wear protective goggles and gloves.

Purification of PCR products or DNA reaction clean-up

For each completed PCR reaction, transfer the aqueous (lower) phase (if mineral oil has been used) to a clean microtube. Perform the purification of PCR product or DNA reaction clean-up based on your sample amount with reagent volumes indicated in Table 2. The purification or clean-up can be performed without heating at step 3 in the protocol.

Concentration of DNA solutions

To concentrate a DNA solution, perform the purification similarly as for PCR product or DNA reaction clean-up based on your sample amount with reagent volumes indicated in Table 2. However, during the elution step use a low volume of elution buffer (5-20 µl) to achieve a concentrated DNA solution.

PROTOCOL

PickPen® tips

The PickPen® 8-Pack (34008) tips are RNase/DNase free and are ready to use (supplied with the 24 preps DEMO kit 71001D)

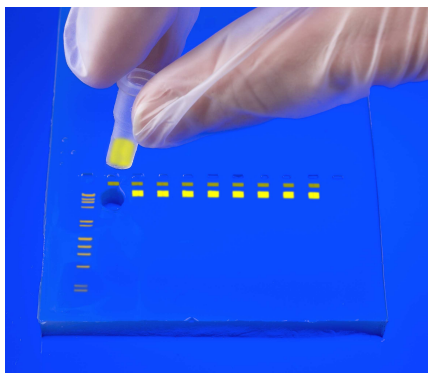
The PickPen® Bulk 96 (34094) tips are untreated and should be autoclaved before use.

Note: Tips should be picked up gently from the pack. Too much pressure may open the pack. The tips packed in bulk quantities in plastic bags are not RNase/DNase free. The tips can be autoclaved (+121 °C at least 20 min) or baked (+180 °C overnight) provided that they are first removed from

the plastic bag or the pack. The separately available PickPen® tip box (34051) can also be autoclaved.

PickO™ gel exciser

The PickO™ gel excisers are presterilized and ready to use. They are packed in bulk quantities in autoclave bags.



NOTES TO THE PROTOCOL:

1. Add ethanol (96-100%) to Wash Buffer 1 (23 ml for 71011D and 140 ml to both bottles for 71001) before use. The volume is also shown on the bottle labels. Mark a tick on the label after ethanol addition. Note: Keep the cap of the bottle tightly closed after ethanol addition to avoid evaporation.
2. Large DNA fragments (>10 kbp) should be handled gently during the different purification steps to avoid DNA degradation. Do not mix by vortexing. Instead mix by finger tapping or inverting the tubes.
3. The volume of Elution Buffer may be adjusted according to the downstream application. DNA fragments can also be eluted into sterile water or TE buffer instead of Elution buffer.
4. Elution can be done at room temperature but the yield may be smaller.
5. The presence of ethidium bromide in the excised gel piece do not reduce the suitability of the purified DNA to the downstream applications

QuickPick™ DNA Fragment purification protocol with PickPen® 1-M

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. PickPen® 1-M magnetic tool. See also the PickPen® instructions.
2. PickPen® Tips.
3. Sterile microtubes.
4. Sterile aerosol resistant micropipettor tips (recommended).
5. Vortex mixer.
6. Heating block.
7. Tube rotator (for microtubes)

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. DNA Fragment Magnetic Particles should be mixed thoroughly just before pipetting. Repeat pipettors should not be used when dispensing magnetic particles.



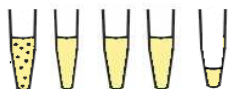
PROTOCOL:

This protocol describes the purification of gel pieces having a weight of 100 mg (see Table 2).

1. **Pipette all the reagents.** This may be done during the gel electrophoresis run to save time.

Number microtubes from 1 to 5 and pipette QuickPick™ DNA Fragment kit reagents into tubes 1 - 5 as follows:

Tube 1:	300 µl	DNA Fragment Gel melting/DNA binding Buffer
	10 µl	DNA Fragment Magnetic Particles
Tube 2:	500 µl	DNA Fragment Wash Buffer 1
Tube 3:	500 µl	DNA Fragment Wash Buffer 1
Tube 4:	500 µl	DNA Fragment Wash Buffer 2
Tube 5:	5-50 µl	DNA Fragment Elution Buffer



1. 2. 3. 4. 5.

2. **After the gel electrophoresis, add the gel slice (see Excision of DNA fragments from agarose gels) into tube 1. Incubate for 5-10 minutes at 50 °C. Make sure that the gel slice dissolves**

completely during the incubation. Mix tube 1 gently by vortexing or manually every two minutes to keep magnetic particles in suspension. Note: Large DNA fragments (>10 kbp) should be handled gently to avoid DNA degradation. Mix by finger tapping or inverting the tubes.

- 3. Remove the tube 1 from 50 °C. Pick up a PickPen® tip with the PickPen® 1-M. Extend the magnet 2-3 times to check that the tip is firmly in place. Collect the magnetic particles from tube 1 with the tool and release them into tube 2 (Wash Buffer 1).** The particles may form a clump which can be gently broken up with the PickPen® tip. Mix the suspension briefly and gently using the tip. Note that the magnet has to be withdrawn at this point. To avoid the degradation of DNA, only gentle mixing is recommended.
- 4. Repeat the washing step in tube 3 (Wash Buffer 1).**
- 5. Collect the magnetic particles from tube 3 with PickPen® and gently dip the magnetic particles in tube 4 (Wash Buffer 2) for 10-30 seconds without releasing the magnetic particles.** The DipWash™ procedure is sufficient to flush away residuals of ethanol which has a detrimental effect on downstream applications. The DipWash™ should be performed with up and down movements of the PickPen® tool without releasing the magnetic particles. A slow circular movement can be performed during the dipping phase.
- 6. For elution, transfer the PickPen® with magnetic particles from tube 4 into tube 5 (Elution Buffer) and release the magnetic particles. Incubate the sample for 5 - 10 minutes at 50°C.** Mix by vortexing or manually every two minutes to keep magnetic particles in suspension. Note: Large DNA fragments (>10 kbp) should be handled gently to avoid DNA degradation. Mix by finger tapping or inverting the tubes.
- 7. Collect the magnetic particles from tube 5 and discard them and the tip.** The eluate in tube 5 containing the purified DNA is ready to be used in downstream applications. If the purified DNA is not used on the same day, store at -20 °C until use.

QuickPick™ DNA Fragment purification protocol with PickPen® 8-M

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. PickPen® 8-M magnetic tool. See also the PickPen® instructions.
2. Sterile 96-well deep well plates.
3. Sterile aerosol resistant micropipettor tips (recommended).
4. Vortex mixer.
5. Heating shaker.
6. Plate shaker.



All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. DNA Fragment Magnetic Particles should be mixed thoroughly just before pipetting. Repeat pipettors should not be used when dispensing magnetic particles. The following instructions are for 8 parallel samples. Samples are transferred into deep well microplates (U-bottom) where the protocol is carried out.

PROTOCOL:

This protocol describes the purification of gel pieces having a weight of 100 mg (see Table 2).

1. **Pipette all the reagents.** This may be done during the gel electrophoresis run to save time.
2. **Pipette QuickPick™ DNA Fragment kit reagents into microplate columns 1 - 5 as follows** (note that each column can hold 8 samples):

Column 1:	300 µl	DNA Fragment Gel melting/DNA binding buffer
	and 10 µl	DNA Fragment Magnetic Particles
Column 2:	500 µl	DNA Fragment Wash Buffer 1
Column 3:	500 µl	DNA Fragment Wash Buffer 1
Column 4:	500 µl	DNA Fragment Wash Buffer 2
Column 5:	5 - 100 µl	DNA Fragment Elution Buffer

3. **Transfer the gel pieces into the respective wells of column 1 (Binding Buffer, Magnetic Particles).** Mix the microplate on the thermoshaker for 5-10 minutes at 50°C. Make sure that the gel slice dissolves completely during the incubation and that the particles are in suspension during this step.
4. **Pick up the PickPen® tips with the PickPen® 8-M.** Extend the magnets 2-3 times to check that the tips are firmly in place. Collect the magnetic particles from column 1 with the tool and

release them into column 2 (Wash Buffer). Mix the suspensions briefly and gently using the PickPen® tips. Note that the magnets have to be withdrawn at this point. To avoid the degradation of DNA only gentle mixing is recommended.

- 5. Repeat the washing step in columns 3 (Wash Buffer 1).**
- 6. Collect the magnetic particles from column 3 with PickPen® 8-M and gently dip the magnetic particles in column 4 (Wash Buffer 2) for 10-30 seconds without releasing the magnetic particles.** The dipping wash is sufficient to flush away residuals of ethanol which has a detrimental effect on downstream applications. The dipping should be performed with up-and down movements of the PickPen® without releasing the magnetic particles. A slow circular movement can be performed during the dipping phase.
- 7. For elution collect the magnetic particles from column 4 with PickPen® and release them into column 5 (Elution Buffer). Mix the microplate on the orbital shaker for 5 - 10 minutes at 50°C.** Make sure that the particles are in a suspension during this step.
- 8. Collect the magnetic particles from column 5 and discard them and the tips.** The eluates in column 5 contain the purified DNA fragments and are ready to be used in downstream applications. If the purified DNA is not used on the same day, store at -20 °C until use.

TROUBLESHOOTING GUIDE

The troubleshooting guide is a tool for solving any problems which may occur during the purification process. BN Products & Services' staff is also always ready to answer any questions that may arise and help you solve your problems.

Possible problem	How to prevent or eliminate the problem
<i>Low or no recovery of DNA</i>	
Agarose gel slice is not completely solubilized	DNA will stay on any insolubilized gel piece. If insoluble gel pieces are observable in the mixture continue the incubation. The incubation temperature for solubilization of gel should be 50 °C. Mix the tube every 2 min during the incubation. Check the amount of Gel melting/DNA binding Buffer (it should be at least 3 x the volume of the gel slice). There is no harm in the case that the Gel melting / DNA binding Buffer volume exceeds the 3 x the volume of the gel slice.
Insufficient mixing or binding time during step 1	Mix the tube every 2 minutes. Precipitated magnetic particles do not get contact with the DNA in the solution. If the volume of liquid in step 1 is more than 1 ml, prolong binding time for 15 minutes.
Ethanol was not added in Wash Buffer 1	Repeat the purification with correctly prepared Wash Buffer 1.
Ethanol concentration is lower than 75 % in the Wash Buffer 1	Be sure that 95–100 % ethanol is used to make up the Wash Buffer 1. Keep the cap of the bottle tightly close during storage.
Too much ethanol from the Wash Buffer 1 remains in the magnetic particle pellet during elution	Make sure that the dip wash during step 6 is long enough. Evaporate the ethanol by incubating the eluate at 50 °C for 10 min.
Sample floats out of the gel slot when loading it into agarose gel	Make sure that the dip wash during step 6 is long enough. Evaporate the ethanol by incubating the eluate at 50 °C for 10 min.
Insufficient amount of Elution Buffer	Increase the amount of Elution Buffer (up to 100 µl)
Inappropriate elution buffer	Use only DNA fragment Elution Buffer, TE-buffer (pH 7-8.5) or sterilized MQ as the elution buffer.

<i>DNA does not perform well in downstream assays</i>	
Ethanol from the Wash Buffer 1 remains in the magnetic particles during DNA elution	Ethanol may decrease the elution efficiency. Before eluting the DNA, remove the ethanol from Wash Buffer 1 with the dip wash during step 6.
Eluate contaminated with agarose gel	The gel is incompletely solubilized in step 1. The incubation temperature for solubilization of gel should be 50 °C. Mix the tube every 2 min during the incubation. Repeat the purification with a more efficient solubilization step.
<i>DNA is shown as a smear when analyzed on agarose gel</i>	
DNA is fragmented	Vigorous mixing and vortexing of DNA above 10 kbp should be avoided during the purification. Mix by finger tapping or inverting of the tubes during the purification process.
DNA sample is overloaded	Smiling and smearing DNA bands are observed if too much DNA is loaded in the well. Reduce the DNA amount per well.

STORAGE AND STABILITY

The QuickPick™ DNA Fragment kit should be stored at room temperature. Magnetic particles should not be frozen.

WARNINGS AND LIMITATIONS

The QuickPick™ DNA Fragment kit is intended for research use only, and is not intended for use in human diagnostic or therapeutic procedures. Standard methods for preventing contamination with DNases during preparation of DNA must be taken. Precautions should also be taken to avoid contamination of opened vials. Do not pipette by mouth.

DNA Fragment Gel Melting/DNA Binding Buffer contains chaotropic salt which is harmful. Appropriate precautions should be taken when handling these solutions.

DISCLAIMERS AND WARRANTIES

BN Products & Services warrants that its products shall be free from defects in materials and workmanship and shall meet performance specifications if stored and used in accordance with the instructions for use, for a period up to the expiry date provided on the kit package. This warranty does not cover normal wear and tear or misuse of the product. *BN Products & Services'* obligation and the purchaser's exclusive remedy under this warranty is limited to replacement, at *BN Products & Services'* expense, of any products defective in manufacture. In no event shall *BN Products & Services* be liable for any special, incidental or consequential damages. This warranty statement may be subject to modification in accordance with local laws, regulations and business practices.

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