ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

2',3'-Cyclic GAMP STING-Based FRET Detection Kit

1 Plate Kit	Catalog Number K081-F1
5 Plate Kit	Catalog Number K081-F5

Species Independent

Sample Types Validated:

Cell Lysates, Tissue Extracts, and Tissue Culture Media

Patent Pending

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

BACKGROUND

2',3'-Cyclic Guanosine monophosphate-adensosine monophosphate (cGAMP) is a second messenger implicated in innate immunity by regulating type I interferon (IFN) responses[1]. When double-stranded DNA is in the cytoplasm of a cell, 2',3'-cGAMP Synthase (cGAS) binds to the DNA activating cGAS to produce 2',3'-cGAMP from ATP and GTP. cGAMP then diffuses and binds to STING (stimulator of interferon genes) completing the cGAS-cGAMP-STING pathway[1-4]. When STING detects the cyclic dinucleotides (CDN's), it induces the TBK1-IRF3 and NF-kB pathways leading to the production of type I interferons and cytokines, thereby playing a role in pathogen detection, metabolic regulation, autoimmunity, and cancer[5-8]. STING binds to 2',3'-cGAMP as well as other CDN's, albeit with lower affinity. This allows it to identify bacterial CDN's directly without the need for cGAS to detect the invasive pathogen's DNA[9-11].

Because it natively binds to 2',3'-cGAMP, STING was used as the base for a biosensor developed by Pollock et al. for in vivo and in vitro testing. Murine STING was chosen as it has demonstrated a higher propensity to bind to bacterial and eukaryotic CDN's versus human STING, lending to potentially broader application. This STING biosensor (BioSTING) is a fluorescence resonance energy transfer (FRET) based sensor designed to detect 2',3'-cGAMP in real-time within living cells. FRET is a fluorescence detection platform based on a distance-dependent relationship between two fluorophores. In the case of BioSTING, mTFP and mKO2 are the fluorophores of choice. When mTFP is excited with a wavelength of 458 nm, the emission is detected at 490 nm when no ligand (2',3'-cGAMP) is present. When BioSTING binds to the ligand, the two fluorophores are positioned in closer proximity to each other, allowing the fluorescence to be transferred from mTFP to mKO2, changing the emission wavelength to 600 nm. Using this detection method, BioSTING shows applicability for in vitro high-throughput screening for CDN production modulation and direct screening for STING agonists and antagonists[12].

RESOURCES

1. Ishikawa, H., et al (2008). STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling, *Nature* 455, 674-678.
2. Wu, J., et al (2013). Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA, *Science*, 339(6121):826-30.
3. Sun, L., et al (2013). Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway, *Science*, 339(6121):786-91.
4. Zhou, W., et al (2018). Structure of the Human cGas-DNA Complex Reveals Enhanced Control of Immune Surveillance, *Molecular Cell*, Vol 174, Issue 2, P300-311.
5. Zhang, C., et al (2019). Structural basis of STING binding with and phosphorylation by TBK1, *Nature,* 567, 394-398.
6. Liu, S., et al (2015). Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation, *Science*, Vol 347, Issue 6227, 10.1126/science.aaa2630.
7. Oliveira Mann, C., et al (2019). Modular Architecture of the STING C-Terminal Tail Allows Interferon and NF-κB Signaling Adaptation, *Molecular Cell*, Vol 27, Issue 4, P1165-1175.
8. Cai, X., et al (2014). The cGAS-cGAMP-STING Pathway of Cytosolic DNA Sensing and Signaling. *Molecular Cell,* Vol 54, Issue 2, P289-296.
9. Shang, G., et al (2019). Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP–AMP, *Nature*, 567, 389-393.
10. Burdette, D., et al (2013). STING is a direct innate immune sensor of cyclic di-GMP. *Nature,* 478, 515-518.
11. Wang, C., et al (2017). Synthesis of All Possible Canonical (3′–5′-Linked) Cyclic Dinucleotides and Evaluation of Riboswitch Interactions and Immune-Stimulatory Effects, J. *Am. Chem.* Soc. 139, 45, 16154-16160.
12. Pollock, A., et al (2020). A STING-based biosensor affords broad cyclic dinucleotide detection within single living eukaryotic cells, *Nat Commun* 11, 3533. doi.org/10.1038/s41467-020-17228-.

ASSAY PRINCIPLE

The 2',3'-Cyclic GAMP (cGAMP) STING-Based FRET Kit is designed to quantitatively measure 2',3'-cGAMP in lysed cells and tissue, as well as tissue culture media samples. Other applications should be carefully evaluated by the end-user.

Please read the complete kit insert before performing this assay. A 2',3'-cGAMP standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. A black microtiter plate to carry out the reaction is provided. Standards or diluted samples are pipetted into the wells. BioSTING, a patented protein that interacts with 2',3'-cGAMP, is added to the standards and samples in the wells to initiate the reaction. After a 15 minute incubation at 37°C with shaking, the plate is read using a microtiter plate capable of reading fluorescent emissions at 490 nm and 600 nm with excitation of 458 nm. Unbound BioSTING will yield a fluorescent signal at 490 nm. When BioSTING binds 2',3'-cGAMP, the distance between the FRET pair fused to STING will close yielding a fluorescent signal at 600 nm. The emission ratio (R) is generated by dividing the signal at 600 nm by the signal at 490 nm. The ratio produced by each standard or diluted sample is then divided by the emission ratio of the zero standard (R0) yielding R/R0. The concentration of 2',3'-cGAMP in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
2',3'-Cyclic GAMP ELISA Kits (Strip Wells and Whole Plate)	K067-H1/H5/H1W/H5W
2',3'-Cyclic GAMP ELISA Kit (384-Well)	K067-H1D
3',3'-Cyclic GAMP ELISA Kits	K073-H1/H5
Acetylcholinesterase Fluorescent Activity Kit	K015-F1
Butyrylcholinesterase Fluorescent Activity Kit	K016-F1
Corticosterone Chemiluminescent ELISA Kits	K014-C1/C5
Corticosterone ELISA Kits (Strip Wells and Whole Plate)	K014-H1/H5/H1W/H5W
Cortisol ELISA Kits (Strip Wells and Whole Plate)	K003-H1/H5/H1W/H5W
Cyclic AMP Direct Chemiluminescent ELISA Kits	K019-C1/C5
Cyclic AMP Direct ELISA Kits	K019-H1/H5
Cyclic cGMP Direct Chemiluminescent ELISA Kits	K020-C1/C5
Cyclic GMP Direct ELISA Kits	K065-H1/H5
Prostaglandin E_2 Multi-Format ELISA Kits (Strip Wells and Whole Plate)	K051-H1/H5/H1W/H5W
Protein Kinase A (PKA) Colorimetric Activity Kit	K027-H1
DMXAA	P024-5MG/25MG
H-151	P023-10MG/50MG
IBMX	P019-100MG/1GM

SUPPLIED COMPONENTS

Black 96 Well Plates

A black plastic microtiter plate(s). See https://www.arborassays.com/assets/PlateDimensions.pdf for plate dimension information.

Kit K081-F1 **or** -F5	1 **or** 5 Each	Catalog Number X025-1EA

2',3'-Cyclic GAMP Standard

2',3'-Cyclic GAMP at 10,000 pmol/mL in a special stabilizing solution.

Kit K081-F1 **or** -F5	125 µL **or** 625 µL	Catalog Number C296-125UL **or** -625UL

DetectX® BioSTING Protein Concentrate

Recombinant FRET protein specific for STING-based interaction with 2',3'-Cyclic GAMP

Kit K08-F1 **or** -F5	1.4 mL **or** 7 mL	Catalog Number C295-1.4ML **or** -7ML

Assay Buffer Concentrate

A 5X concentrate that must be diluted with deionized or distilled water.

Kit K081-F1 **or** -F5	28 mL **or** 55 mL	Catalog Number X157-28ML **or** -55ML

Plate Sealer

Kit K081-F1 **or** -F5	1 **or** 5 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must be stored at -80°C.

Once opened, the kit can be store at 4°C except the BioSTING Protein Concentrate. **The BioSTING Protein Concentrate must be stored at -80°C.** Upon first use, aliquot unused protein to prevent unnecessary freeze-thaw cycles.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25 µL.

A microplate shaker capable of incubating at 37°C.

Fluorescent 96 well microplate reader capable of reading fluorescent emission at 600 nm and 490 nm, with excitation at 458 nm. Please contact your plate reader manufacturer for suitable filter sets.

Software for converting raw relative fluorescent unit (FLU) readings from the plate reader. Set plate parameters for a 96-well Corning Costar 3650 plate. Download a sample calculation template for standard curve generation and sample calculations at www.ArborAssays.com/resources.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Buffers, including other manufacturers, containing oxidative agents or EDTA will inhibit FRET reaction. Make sure all buffers used for samples are free of oxidizing agents and chelators.

SAMPLE TYPES

This assay has been tested and validated for lysed cells, tissue lysate, and tissue culture media. Matrix interference has been evaluated for serum, urine, and saliva as it is unknown if 2',3'-cGAMP is present in these matrices. Samples should be stored at ≤-70 ℃ for long term storage. Samples containing visible particulate should be centrifuged prior to use.

SAMPLE PREPARATION

The kit is designed for the detection of endogenous 2',3'-Cyclic GAMP across a wide range of tissues and cell lines. However, until a sample is tested, undetectable levels of 2',3'-cGAMP cannot be excluded. It is up to the end user to determine if their sample type has measurable levels of the analyte, as well as the suitability of their lysis buffer (if appropriate).

Samples may need to be extracted depending on source. We suggest a study of the literature to determine suitable methods of isolating cGAMP from tissues and cells.

Tissue Samples

Tissues samples should be frozen in liquid nitrogen and stored at -80°C if analysis is not to be carried out immediately. Grind the frozen tissue in a stainless steel mortar under liquid nitrogen until it is a fine powder. Allow the liquid nitrogen to evaporate and weigh the powdered tissue. Lyse cells further through mechanical means in assay buffer and then centrifuge at ≥ 600 x g at 4°C for 15 minutes. Alternatively, the end user can use an appropriate lysis buffer after testing for lysis buffer compatibility (refer to interferents on page 14). Collect the supernatant and run in the assay directly after dilution into Assay Buffer or off a standard curve generated in lysis buffer if shown to be compatible

Cells

Harvest cells by centrifugation and wash once with ice-cold PBS. Resuspend cell pellets in ice-cold 80% methanol and incubate on ice for 20 min. Lyse cells further by sonication. Centrifuge and dry cellular extracts under vacuum. Resuspend in Assay Buffer before use or store at −20 °C.

Pollock, A., et al (2020). A STING-based biosensor affords broad cyclic dinucleotide detection within single living eukaryotic cells, Nat Commun 11, 3533. doi.org/10.1038/s41467-020-17228-.

Tissue Culture Media

For measuring 2',3'-cGAMP in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Use all samples within 2 hours or store frozen at ≤ -70˚C.

REAGENT PREPARATION

Allow the kit reagents to thaw and come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted, this is stable for 3 months at 4°C.

BioSTING Protein

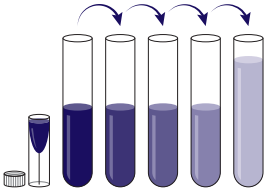
Dilute BioSTING Protein Concentrate 1:2 by adding one part of the concentrate to one part of Assay Buffer according to the table below:

	1/2 Plate	One Plate
BioSTING Protein Concentrate	0.7 mL	1.4 mL
Assay Buffer	0.7 mL	1.4 mL

Use BioSTING protein 1x within 1 hour preparation.

Standard Preparation

Label test tubes as #1 through #7. Pipet 950 µL of Assay Buffer into tube #1 and 200 µL of Assay Buffer into tubes #2 to #7. **Prerinse the pipet tip several times to ensure accurate delivery of the 2',3'Cyclic GAMP stock.** Carefully add 50 µL of the stock solution to tube #1 and vortex completely. Take 800 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of 2',3'-Cyclic GAMP in tubes 1 through 7 will be 500, 400, 320, 256, 204.8, 163.8 and 131.1 pmol/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	**950**	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**50**	800	800	800	800	800	800
Final Conc (pmol/mL)	500	400	320	256	204.8	163.8	131.1

Use Standards within 1 hour of preparation.

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine 2',3'-cGAMP concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.
2. Preheat microplate shaking incubator to 37°C.
3. Pipet 75µL standards or samples into wells across the plate rows (1 to 12).
4. Pipet 75 µL Assay Buffer into the zero standard wells.
5. Add 25 µL of the diluted DetectX® 2',3'-cGAMP BioSTING Protein to each well using a repeater pipet.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at 900 rpm 37°C for 15 minutes.
7. Read the flourescent signal generated from each well in a plate reader capable of reading the flourescent signal at 600 nm and 490 nm with excitation at 458 nm.
8. Use the Excel template plate available on our resource page to calculate 2',3'-cGAMP concentration for each sample.

CALCULATION OF RESULTS

Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data after taking the 600 nm/490 nm average FLU ratio of each standard and dividing by the FLU ratio of the zero.

The sample concentrations obtained, calculated from the Standard curve R/R0, should be multiplied by the dilution factor (if any) to obtain neat sample values.

Alternatively go to our website and download a sample concentration spreadsheet at www.ArborAssays.com/resources.

Or use the online tool from MyAssays to calculate the data: Coming Soon.

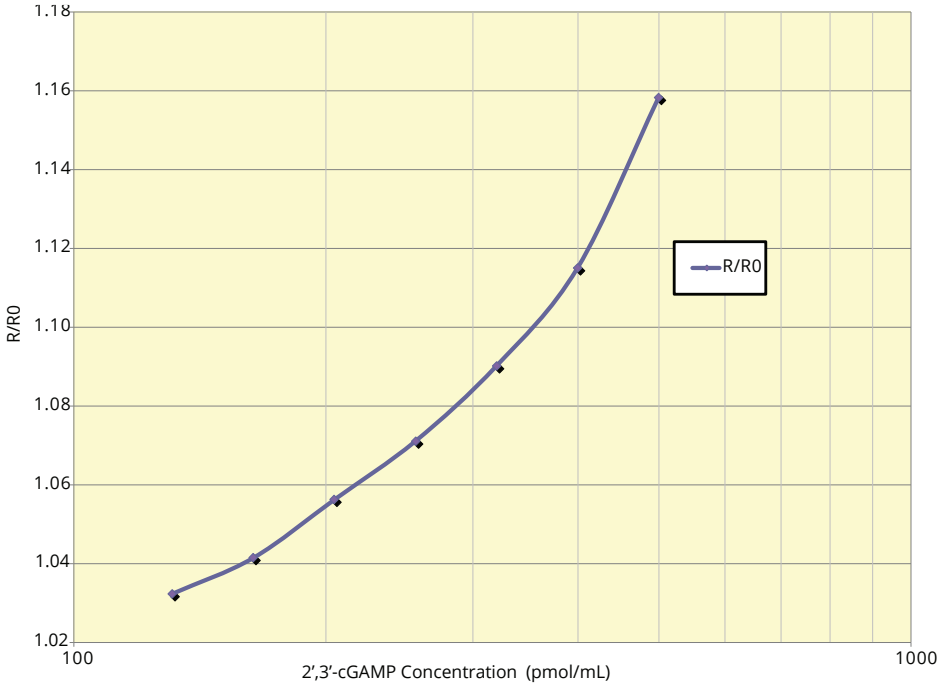
TYPICAL DATA

Sample	Mean FLU 600 nm	Mean FLU 490 nm	Mean Ratio (R) (600 nm / 490 nm)	R/R0	2',3'-Cyclic GAMP Concentration (pmol/mL)
Zero	30,556	17,776	1.719		0
Standard 1	33,247	16,699	1.991	1.158	500
Standard 2	32,850	17,139	1.917	1.115	400
Standard 3	32,601	17,396	1.874	1.090	320
Standard 4	32,517	17,661	1.841	1.071	256
Standard 5	32,415	17,852	1.816	1.056	204.8
Standard 6	32,348	18,068	1.790	1.042	163.8
Standard 7	32,161	18,122	1.775	1.032	131.1
Sample 1	33,764	17,338	1.947	1.133	439.0
Sample 2	32,840	17,801	1.845	1.073	260.1

Always run your own standard curve for calculation of results. Do not use this data.

Conversion Factor: 0.1 pmol/mL of cGAMP is equivalent to 71.8 pg/mL.

Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The sensitivity was determined at two (2) standard deviations from the zero standard along the standard curve.

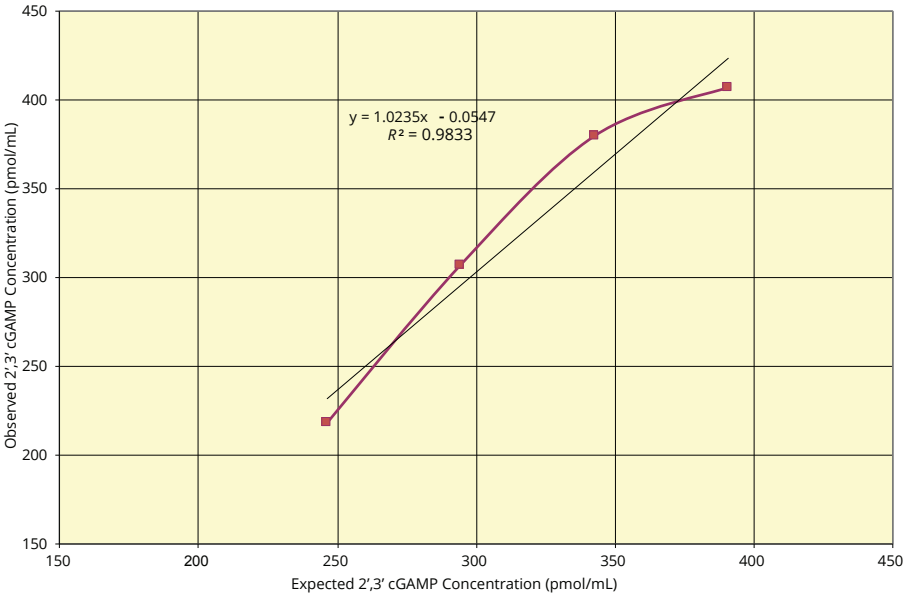
Sensitivity was determined as 82.02 pmol/mL.

VALIDATION DATA

Linearity

Linearity was determined by taking two diluted samples, one spiked with a low cGAMP level of 198.1 pmol/mL and one spiked with a higher level of 439.2 pmol/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Sample	Low Sample	Expected Conc. (pmol/mL)	Observed Conc. (pmol/mL)	% Recovery
80%	20%	391.0	407.2	104.2
60%	40%	342.7	379.9	110.8
40%	60%	294.5	307.3	104.3
20%	80%	246.3	218.1	88.5
			Mean Recovery	**102.0%**



Intra Assay Precision

Three samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated cGAMP concentrations were:

Sample	2',3'-Cyclic GAMP Conc. (pmol/mL)	%CV
1	458.5	6.2
2	256.8	11.6
3	168.6	10.7

Inter Assay Precision

Two samples were diluted with Assay Buffer and run in duplicates in twenty assays run over multiple days by multiple operators. The mean and precision of the calculated cGAMP concentrations were:

Sample	2',3'-Cyclic GAMP Conc. (pmol/mL)	%CV
1	458.5	9.0
2	264.8	8.7

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated on the standard curve.

Nucleotide	Cross Reactivity (%)
2',3'-cGAMP	100
2',3'-c-di-AMP2 (synthetic)	73.35
2',2'-cGAMP (synthetic)	34.03
3',3'-cGAMP (bacterial)	25.92
c-di-AMP	16.04
c-di-GMP	4.04
cAMP	< 0.01
AMP	< 0.01
cGMP	< 0.01
GMP	< 0.01
ATP	< 0.01
GTP	< 0.01

INTERFERENTS

A variety of detergents at multiple dilutions were tested as possible interfering substances in the assay when comparing concentrations to those generated by Assay Buffer spiked sample.

Addition	% Maximum Dose	% Change
SDS	0.00025	13.0
NP-40	0.25	-2.6
Tween-20	0.25	3.2
EDTA	0.00025	2.78
DMSO	0.5	-3.97
M-PER	1:20	15.92
X050-100ML Lysis Buffer	1:20	13.06

Commercially available lysis buffers (RIPA and M-PER™) were spiked, diluted and tested in the assay along with a spiked control in Assay Buffer. These buffers showed interference even with a 1:20 dilution in Assay Buffer. These lysis buffers should not be used. It is up to the end user to determine if their lysis buffer is suitable.

SAMPLE VALUES

Human lung tumor lysate was spiked with standard and diluted in Assay Buffer. Concentrations were compared to a similarly spiked control of Assay Buffer. Recovery for lung tumor tissue lysate diluted 1:70-1:80 averaged 101.8%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with ELISA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

FSC Printed on Forest Stewardship Council certified paper