Clean Total RNA Kit

Instructions for Use

V.3 - NOVEMBER 2024

For Research Use Only

REF **CTR-R0096, CTR-R0384**

CleanNA, Coenecoop 75, 2741 PH, Waddinxveen, the Netherlands

Information in this document is subject to change without notice.

Disclaimer

For Research Use Only. Not for use in diagnostic procedures.

CleanNA disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. To the fullest extend allowed by law, in no event shall CleanNA be liable, whether contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequental damages in connection with or arising from this document, including, but not limited to the use thereof, whether or not forseeable and whether or not CleanNA is advised of the possibility of such damages.

Trademarks

The trademarks mentioned herein are the property of their respective owners.

Contents

Introduction and Principle

The Clean Total RNA Kit allows for isolation of high-quality total cellular RNA from a wide variety of tissue samples and cultured cells. Total RNA can be isolated from 5-10 mg tissue or 1 x 10^6 cultured cells.

To become familiar with all steps and procedures, please read this user manual carefully when performing the RNA isolation procedure for the first time.
Samples are lysed using the DHX Lysis combined with Proteinase K. During lysis, proteinase K will digest the proteins in the samples. With addition of ethanol and our CleanNA Particles HS both DNA and RNA are bound. After 2 wash steps, the DNA and RNA will be eluted from the CleanNA Particles HS. Genomic DNA is then removed with a DNase I digestion step after which the RNA will be rebound to the CleanNA Particles HS. After two quick wash steps, RNA is eluted.

The isolated RNA is directly suitable for use in downstream applications such as RT-PCR, restriction digestion, and hybridization applications.

Since the Clean Total RNA Kit uses CleanNA's paramagnetic beads, it can also be adapted to our CleanXtract 96 or liquid handlers including Beckman Coutler's Biomek FX, Hamilton Star, Dynamic Devices, Tecan Genesis and Thermo Kingfisher Flex instruments.

Materials Provided

Kit Contents:

Component	CTR-R0096 (96 preps)	CTR-R0384 (384 preps)
DHX Lysis*	50 mL	200 mL
Proteinase K (20 mg/ml)	2.2 mL	9 mL
CleanNA Particles HS	2.2 mL	8.8 mL
AR Wash*	22 mL	88 mL
CR Wash	50 mL	3 x 50 mL
DNase Digestion Buffer	25 mL	25 mL
CleanNA DNase I	220 µL	4 x 220 µL
DH Binding	20 mL	65 mL
RNA Elution Buffer	25 mL	100 mL

⚠ **Note:** Check all buffers for precipitates prior to usage. Any precipitates can be re-dissolved by warming the buffer(s) to 37ºC and shaking gently.

Reagent Shipping and Storage

Shipping of the Clean Total RNA Kit should be done at room temperature (15-25 °C).

Do not freeze the components of the Clean Total RNA Kit that do not have a storage temperature of -20ºC.

Component	Storage
DHX Lysis	15-25ºC
Proteinase K (20 mg/ml)	15-25ºC (for storage > 12 months, store at 2-8°C)
CleanNA Particles HS	2-8ºC
AR Wash	15-25ºC
CR Wash	15-25ºC
DNase Digestion Buffer	-20ºC
CleanNA DNase I	-20ºC
DH Binding	-20ºC
RNA Elution Buffer	15-25ºC

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

The LOT numbers on the CleanNA Particles HS box packaging and freezer components bag are different from the LOT number on the CleanNA Particles HS bottle. The LOT numbers on the box/bag match the LOT number of the whole kit and the one on the bottles is specifically for the particles/freezer components. Since the CleanNA Particles HS and freezer components are stored at a different temperature, please make sure that the LOT number on the box/bag packaging matches the LOT number of the kit before use.

Precautions

For all safety information, please consult the safety data sheet (SDS).

DHX Lysis	
 	Harmful if swallowed. Harmful to aquatic life with long lasting effects. Wash all exposed external body areas thoroughly after handling. Do not eat, drink or smoke when using this product. Avoid release to the environment. **If in eyes:** Wash eyes with fresh running water, seek medical attention without delay. **If swallowed:** Rinse mouth. Do NOT induce vomiting. **If on skin:** Remove all contaminated clothing and footwear and flush skin and hair with running water. **If inhaled:** Remove from contaminated area.
AR Wash	
	Causes serious eye irritation. Harmful if swallowed. Causes skin irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/protective clothing/eye protection/face protection. **If in eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing **If swallowed:** Call a POISON CENTER or doctor/physician if you feel unwell. **If on skin:** Wash with plenty of water and soap. **If inhaled:** Remove from contaminated area, and lay patient down.
Proteinase K (20 mg/ml)	
	May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing mist/vapours/spray. n case of inadequate ventilation wear respiratory protection. **If in eyes:** Immediately wash eyes with water. **If swallowed:** Immediately give a glass of water. **If on skin:** Remove contaminated clothing and footwear and flush skin and hair with running water. **If inhaled:** Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Note: For safe disposal, please consult your local waste regulations.

Quality Control

CleanNA produces each lot of Clean Total RNA Kit according to specific predetermined and validated protocols in the Quality Management System (QMS). CleanNA's QMS is EN-ISO 13485 certified.

Materials and Equipment to be Supplied by User

Tissue protocol

Materials and Reagents to be supplied by user for the Tissue protocol:

- Ethanol absolute
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-96-RN50)
- Centrifuge with swing-bucket rotor capable of 4,000 x g including an adaptor for 96-well plates
- 96-well microplates with a minimum capacity of 1 mL (e.g. ABgene AB-1227 or AB-0661)
- Vortexer
- Multichannel pipettes and nuclease-free pipette tips
- Multichannel reagent reservoirs
- Sealing film
- Equipment for disrupting tissue (MM300 Mixer Mill or Geno/Grinder 2000/2010) or mortar and pestle

Cultured Cells protocol

Materials and Reagents to be supplied by user for the Cultured Cells protocol:

- Ethanol absolute
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-96-RN50)
- Centrifuge with swing-bucket rotor capable of 4,000 x g including an adaptor for 96-well plates
- 96-well microplates with a minimum capacity of 1 mL (e.g. ABgene AB-1227 or AB-0661)
- Vortexer
- Multichannel pipettes and nuclease-free pipette tips
- Multichannel reagent reservoirs
- Sealing film

- Equipment for disrupting tissue (MM300 Mixer Mill or Geno/Grinder 2000/2010) or mortar and pestle
- Pre-chilled PBS is using cells grown in a monolayer
- Trypsin if using cells grown in a monolayer

Working RNase free

When using the Clean Total RNA Kit it is important to work RNase free. RNases are present everywhere and general precautions should be taken to avoid the introduction of RNases and other contaminating nucleases while working with the Clean Total RNA Kit. The most common sources of RNase while working with RNA samples are hands, dust particles and contaminated laboratory solutions, equipment and glassware. To minimize the risk of RNase contamination we recommend the following precautions:

- Always use gloves when handling RNA samples. Change your gloves frequently to avoid contaminations
- Ensure to use RNase free filter tips for pipetting
- Use materials such as disposable consumables, which are guaranteed RNase free
- Use reagents which are guaranteed RNase free. Creating aliquots from buffers lowers the risk of RNase contamination in buffers, reagents, etc.
- Avoid using reagents, consumables and equipment dedicated for common use or general lab processes
- If possible and available, work in a separate room, fume hood or lab space
- Clean all working surfaces with commercial RNase inhibiting surfactant or 70% ethanol before starting your work / experiment

Preparation of Reagents

Prepare all materials required before starting to minimize RNA degradation. Wear gloves/protective goggles and take great care when working with chemicals.

AR Wash

Dilute AR Wash with ethanol absolute as follows and store at room temperature.

Kit	Ethanol Absolute to be Added
CTR-R0096	28 mL
CTR-R0384	112 mL

CR Wash

Dilute CR Wash with ethanol absolute as follows and store at room temperature.

Kit	Ethanol Absolute to be Added
CTR-R0096	200 mL
CTR-R0384	200 mL per bottle

Lysis mastermix

Prepare a mastermix of DHX Lysis and Proteinase K (20 mg/ml) according to the table below.

Component	Amount per prep	Total Amount per 96-well plate
DHX Lysis	450 µL	47.5 mL*
Proteinase K (20 mg/ml)	20 µL	2.1 mL*

* 10% excess volume has been calculated for a 96-well plate.

96-Protocol for Tissue

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on page 10
- Before use, vortex the CleanNA Particles HS thoroughly.

Protocol:

1. Prepare a mastermix of DHX Lysis and Proteinase K (20 mg/ml) according to the table below.

Component	Amount per prep	Total Amount per 96-well plate
DHX Lysis	450 µL	47.5 mL*
Proteinase K (20 mg/ml)	20 µL	2.1 mL*

* 10% excess volume has been calculated for a 96-well plate.

2. Choose one of the two following methods for homogenization of samples:

A. Mechanical tissue disruption

a) Place sample and appropriate steel beads into a stainless steel grinding plate.
b) Add 470 µL DHX Lysis/Proteinase K (20 mg/ml) mastermix to the samples.
c) Grind sample at 30 Hz for 1-2 minutes according to manufacturer's instructions (For GenoGrinder 2000/2010, grind at 1500 RPM for 2 minutes).
e) Remove the plate from the homogenizer and remove the caps.

⚠ **Note:** It may be necessary to centrifuge the plate briefly to remove debris from the caps.

B. Manual sample preparation

a) Prepare the sample by collecting fresh tissue sample in a 30 mL mortar.
b) Freeze the samples by dipping in liquid nitrogen using tweezers or tongs to fill the tube.
c) Grind the tissue using a clean pestle.
d) Transfer the grounded powder and liquid nitrogen into 96- well deep-well plate and allow the liquid nitrogen to evaporate.
e) Add 470 µL DHX Lysis/Proteinase K (20 mg/ml) mastermix.

3. Centrifuge at 4,000 x g for 5 minutes.
4. Transfer 400 µL lysate to a 96-well plate. Do not disturb the debris pellet.

⚠ **Note:** The 96-well plate must have minimum volume of 1 mL and be compatible with the magnetic stand used.

5. Add 300 µL ethanol absolute and 20 µL CleanNA Particles HS to each sample.

⚠ **Note:** During storage the CleanNA Particles HS will settle and clump together at the bottom of the bottle. Ensure to vortex the CleanNA Particles HS thoroughly before use. CleanNA Particles HS and ethanol can be made as a mastermix.

6. Vortex the samples for 5 minutes at room temperature.

7. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

9. Remove the plate from the magnetic separation device.

10. Add 400 µL AR Wash to each sample.

⚠ **Note:** AR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

11. Resuspend the CleanNA Particles HS by vortexing for 3 minutes or pipetting up and down 20 times.

⚠ **Note:** Complete resuspension is required for adequate washing of the CleanNA Particles HS.

12. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

13. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL CR Wash to each sample.

⚠ **Note:** CR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

16. Resuspend the CleanNA Particles HS by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** Complete resuspension is required for adequate washing of the CleanNA Particles HS.

17. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

18. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

⚠ **Note:** All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

19. Incubate at room temperature for 5 minutes to dry the samples. Remove residual liquid with a pipettor.

20. Remove the plate from the magnetic separation device.

21. Add 100 µL RNA Elution Buffer to each sample.

22. Vortex at room temperature for 5 minutes.

23. Prepare the CleanNA DNase I mix according to the table below.

Component	Amount per prep	Total Amount per 96-well plate
DNase Digestion Buffer	50 µL	5.28 mL*
CleanNA DNase I	2 µL	211 µL*

* 10% excess volume has been calculated for a 96-well plate.

24. Add 52 µL CleanNA DNase I mix to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads.

25. Incubate at room temperature for 10 minutes.

26. Add 150 µL DH Binding to each sample. Mix by vortexing for 1 minute.

27. Add 300 µL CR Wash to each sample.

⚠ **Note:** CR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

28. Vortex the samples for 10 minutes.

29. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

30. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

31. Remove the plate from the magnetic separation device.

32. Add 400 µL CR Wash to each sample.

⚠ **Note:** CR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

33. Resuspend the CleanNA Particles HS by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** Complete resuspension is required for adequate washing of the CleanNA Particles HS.

34. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

35. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

36. Leave the plate on the magnetic separation device for 10 minutes to air dry the CleanNA Particles HS. Remove any residual liquid with a pipettor.

⚠ **Note:** All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

37. Remove the plate from the magnetic separation device.

38. Add 50-100 µL RNA Elution Buffer to each sample.

39. Resuspend the CleanNA Particles HS by vortexing for 5 minutes.

⚠ **Note:** Complete resuspension is required for efficient elution.

40. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

41. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided).

42. Store RNA at -80°C.

96-Protocol for Cultured Cells

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on page 10
- Before use, vortex the CleanNA Particles HS thoroughly

Protocol:

1. Prepare a mastermix of DHX Lysis and Proteinase K (20 mg/ml) according to the table below.

Component	Amount per prep	Total Amount per 96-well plate
DHX Lysis	450 µL	47.5 mL*
Proteinase K (20 mg/ml)	20 µL	2.1 mL*

* 10% excess volume has been calculated for a 96-well plate.

2. Harvest cells by choosing one of the following methods:

A. For cells grown in suspension

a) Determine the number of cells. Do not use more than 1 x 10^6 cells.
b) Pellet the appropriate number of cells by centrifuging at 500 x g for 5 minutes.
c) Add 470 µL DHX Lysis/Proteinase K (20 mg/ml) mastermix to each sample.
d) Pipet up and down several time to mix the samples.

B. For cells grown in a monolayer

These cells can either be lysed directly in the cell culture dish or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell culture flasks should always be trypsinized.

Direct cell lysis:
a) Determine the number of cells.
b) Aspirate and discard the cell culture medium completely.
c) Add 470 µL DHX Lysis/Proteinase K (20 mg/ml) mastermix to each sample.
d) Pipet up and down several time to mix the samples.

Trypsinization of cells:
a) Determine the number of cells.
b) Aspirate and discard the cell culture medium completely.
c) Wash cells with 4°C PBS.
d) Aspirate and discard the PBS.

e) Wash cells with 4°C PBS containing 0.1-0.25% trypsin.
f) Check cells for detachment. Make sure cells are detached before proceeding.
g) Add cell culture medium containing serum to inactivate the trypsin.
h) Transfer cells to an RNase-free microplate.
i) Centrifuge at 500 x g for 5 minutes.
j) Aspirate and discard the supernatant completely.
k) Add 470 µL DHX Lysis/Proteinase K (20 mg/ml) mastermix to each sample.
l) Pipet up and down several time to mix the samples.

3. Centrifuge at 4,000 x g for 5 minutes.

4. Transfer 400 µL lysate to a 96-well plate. Do not disturb the debris pellet.

⚠ **Note:** The 96-well plate must have minimum volume of 1 mL and be compatible with the magnetic stand used.

5. Add 300 µL ethanol absolute and 20 µL CleanNA Particles HS to each sample.

⚠ **Note:** During storage the CleanNA Particles HS will settle and clump together at the bottom of the bottle. Ensure to vortex the CleanNA Particles HS thoroughly before use. CleanNA Particles HS and ethanol can be made as a mastermix.

6. Vortex the samples for 5 minutes at room temperature.

7. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

9. Remove the plate from the magnetic separation device.

10. Add 400 µL AR Wash to each sample.

⚠ **Note:** AR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

11. Resuspend the CleanNA Particles HS by vortexing for 3 minutes or pipetting up and down 20 times.

⚠ **Note:** Complete resuspension is required for adequate washing of the CleanNA Particles HS.

12. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

13. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL CR Wash to each sample.

⚠ **Note:** CR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

16. Resuspend the CleanNA Particles HS by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** Complete resuspension is required for adequate washing of the CleanNA Particles HS.

17. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

18. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

⚠ **Note:** All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

19. Incubate at room temperature for 5 minutes to dry the samples. Remove residual liquid with a pipettor.

20. Remove the plate from the magnetic separation device.

21. Add 100 µL RNA Elution Buffer to each sample.

22. Vortex at room temperature for 5 minutes.

23. Prepare the CleanNA DNase I mix according to the table below.

Component	Amount per prep	Total Amount per 96-well plate
DNase Digestion Buffer	50 µL	5.28 mL*
CleanNA DNase I	2 µL	211 µL*

* 10% excess volume has been calculated for a 96-well plate.

24. Add 52 µL CleanNA DNase I mix to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads.

25. Incubate at room temperature for 10 minutes.

26. Add 150 µL DH Binding to each sample. Mix by vortexing for 1 minute.

27. Add 300 µL CR Wash to each sample.

⚠ **Note:** CR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

28. Vortex the samples for 10 minutes.

29. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

30. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

31. Remove the plate from the magnetic separation device.

32. Add 400 µL CR Wash to each sample.

⚠ **Note:** CR Wash must be diluted with ethanol absolute prior to use. Please see Page 10 for instructions.

33. Resuspend the CleanNA Particles HS by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** Complete resuspension is required for adequate washing of the CleanNA Particles HS.

34. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

35. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles HS.

36. Leave the plate on the magnetic separation device for 10 minutes to air dry the CleanNA Particles HS. Remove any residual liquid with a pipettor.

⚠ **Note:** All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

37. Remove the plate from the magnetic separation device.

38. Add 50-100 µL RNA Elution Buffer to each sample.

39. Resuspend the CleanNA Particles HS by vortexing for 5 minutes.

⚠ **Note:** Complete resuspension is required for efficient elution.

40. Place the plate on the magnetic separation device to magnetize the CleanNA Particles HS. Incubate at room temperature until the CleanNA Particles HS are completely cleared from solution.

41. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided).

42. Store RNA at -80°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Low RNA Yield	Incomplete resuspension of CleanNA Particles HS	Resuspend the CleanNA Particles HS by vortexing before use.
	Ethanol was not added to CR Wash	Add ethanol absolute to CR Wash as instructed on page 10.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	CleanNA Particles HS not resuspended during binding	Vortex vigorously for 2 minutes after addition of ethanol and CleanNA Particles HS.
	Loss of CleanNA Particles HS during procedure	Be careful not to remove the CleanNA Particles HS during the procedure.
	RNases present while performing the isolation	Ensure to work RNase free, see page 10 for more information.
Problem with downstream application	Insufficient RNA was used	RNA in the sample already degraded. Do not freeze/thaw the sample more than once. Do not store at room temperature.
Carryover of the CleanNA Particles HS in the elution	Carryover of the CleanNA Particles HS in the eluted RNA will not affect downstream applications.	To remove any carryover CleanNA Particles HS from the eluted RNA, simply place the plate on the magnetic separation device and wait until the eluate has cleared. Carefully transfer the RNA eluate to a new 96-well plate.

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Total RNA Kit (96 Preps)	CTR-R0096
Clean Total RNA Kit (384 Preps)	CTR-R0384

Product	Part Number
Clean Magnet Plate 96-well RN50	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
3	29/NOV/2024	Reagent shipping and storage	Change in RNA Elution buffer storage temperature
2	03/MAY/2024	Total revision	Updated buffer names and Proteinase K volume.
1	06/FEB/2023	Initial version	Initial version

Contact

Coenecoop 75 | 2741 PH Waddinxveen | The Netherlands
T: +31 (0) 182 22 33 50 | F: +31 (0) 182 22 33 98 | info@cleanna.com
www.cleanna.com