

# AGENCOURT<sup>®</sup> FORMAPURE<sup>®</sup> KIT

## NUCLEIC ACID ISOLATION FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE

Please refer to <http://www.agencourt.com/technical> for updated protocols and refer to MSDS instructions <http://www.beckmancoulter.com/customersupport/msds/msds.asp> when handling or shipping any chemical hazards. For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186.

AGENCOURT FORMAPURE is a trademark of Agencourt Bioscience and is for laboratory use only.

### **Agencourt FormaPure: Table of Contents**

Introduction.....	1
Kit Specifications.....	1
Working Under RNase Free Conditions.....	2
Materials Supplied in the Kit.....	2
Total Nucleic Acid Extraction - Process Overview.....	2
Total Nucleic Acid Extraction - Materials Supplied by the User.....	2
Total Nucleic Acid Extraction.....	2
RNA Only - Process Overview.....	2
RNA Only - Materials Supplied by the User.....	2
RNA Only Extraction Using Agencourt FormaPure 96-well & Tube Protocol.....	2

### **Introduction**

The Agencourt FormaPure nucleic acid purification kit utilizes the patented Agencourt SPRI<sup>®</sup> paramagnetic bead-based technology to isolate total nucleic acids (both DNA & RNA) from a maximum input 5 sections of 10µm slices of formalin-fixed, paraffin-embedded tissue. The protocol can be performed in both 96-well plates (manually and fully automated) and in 1.5 mL tubes. Nucleic acid extraction begins with the addition of a reagent that melts paraffin and de-crosslinks nucleic acids. Proteinase K is then added to complete tissue digestion and inactivate nucleases. Next, binding buffer is added to facilitate immobilization of the nucleic acids to the surface of paramagnetic beads. The contaminants are rinsed away using a simple washing procedure. The products are then eluted with water. The Agencourt FormaPure procedure does not require vacuum filtration or centrifugation.

### **Kit Specifications**

The Agencourt FormaPure kit is manufactured under RNase-free conditions and has been tested and certified not to contain contaminating nucleases.



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**Working Under RNase Free Conditions**

RNases are ubiquitous and general precautions should be followed in order to avoid the introduction of contaminating nucleases during the Agencourt FormaPure procedure. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions and glassware. When working with RNA, the following procedures should be followed to limit RNase contamination:

- Always work with gloved hands and change gloves frequently
- Use RNase free, filtered pipette tips for pipetting whenever possible
- Use plastic, disposable consumables that are certified RNase free
- Use dedicated RNase free equipment, e.g. pipettes, pipette tips, gels boxes, etc.
- Avoid using reagents, consumables and equipment that are in common use for other general lab processes
- When available, work in a separate room, fume hood or lab space
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contaminating the stock solution
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% ethanol before starting work

**Materials Supplied in the Kit**

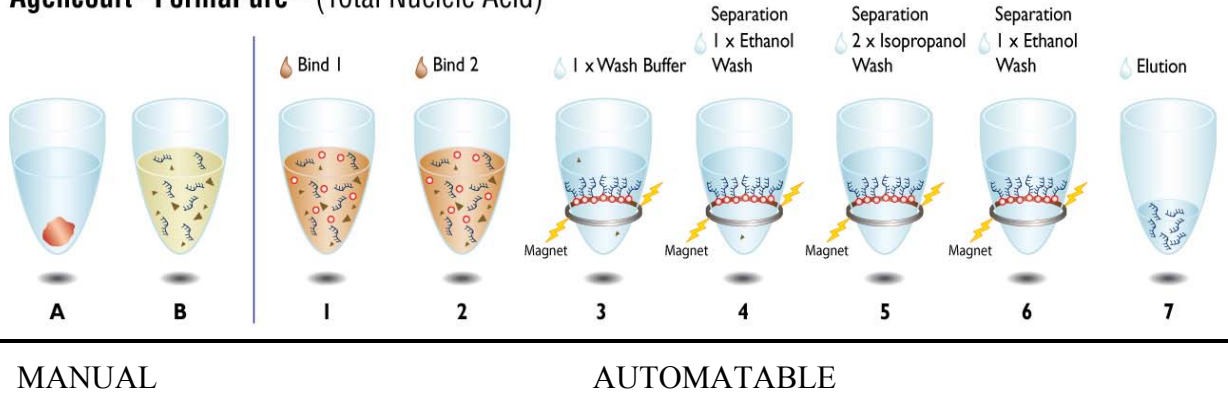
Reagent	Storage Conditions on Arrival	Storage Conditions once In Use
Agencourt FormaPure Lysis Buffer	Room Temperature	Room Temperature
Agencourt FormaPure Bind 1 Buffer	Room Temperature	Room Temperature
Agencourt FormaPure Bind 2 Buffer	4°C	Prepare fresh
Agencourt FormaPure Wash Buffer	Room Temperature	Room Temperature*
PK (Proteinase K)	-20°C	-20°C**
PK Buffer	Room Temperature	-20°C***

\* with isopropanol \*\* with PK Buffer \*\*\* with Proteinase K

**For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186**

**Total Nucleic Acid Extraction - Process Overview**

**Agencourt® FormaPure™ (Total Nucleic Acid)**



The Agencourt FormaPure procedure for total nucleic acid, is performed in the following stages:

**A & B.** Digestion of tissue and decrosslinking of nucleic acids

1. Addition of Bind 1 Buffer to lysate
2. Addition of Bind 2 Buffer to lysate
3. Separation of beads and washing of captured products with wash buffer
4. Washing with 70% ethanol to remove contaminants
5. Washing with 90% isopropanol
6. Washing with 70% ethanol
7. Elution of nucleic acids from the magnetic particles

**Total Nucleic Acid Extraction - Materials Supplied by the User**

*Consumables and Hardware:*

- Agencourt Supermagnet Ring Magnetic Plate (Agencourt product # 000322; <http://www.agencourt.com>) or SPRISand for 1.7 mL tubes ( Agencourt product # 001139)
- Microtome for tissue sectioning
- **For Tube Format:**
  - 1.7 mL microcentrifuge tubes (ABGene Cat # T6050G; <http://www.abgene.com>)

**For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186**

- **For Plate Format:**
  - **For Manual Digestion/Processing 1.2 mL plates** (ABGene Cat # AB1127; <http://www.abgene.com>)
  - Adhesive plate film (ABGene Cat # AB-0558)
- 70-72°C and 55°C water bath

Reagents:

- 100% Isopropanol, ultra pure (American Bioanalytical product # AB-07015; <http://www.americanbio.com/>)
- Fresh 70% ethanol made with nuclease free water (*Note: Prepare 70% ethanol fresh for optimal results*) (American Bioanalytical product # AB-00138; <http://www.americanbio.com/>)
- Reagent grade water, nuclease-free (Ambion product # 9932; <http://www.ambion.com>)

**Total Nucleic Acid Extraction**

*Note:* For RNA Only Extraction, follow alternative protocol starting on page 9.

**Before you start**

- **Preheat water baths to 72°C and 55°C.**
- **Ensure that Agencourt FormaPure Proteinase K and Wash Buffer have been assembled according to the following instructions:**
  - **Proteinase K (40 mg/mL):** Add PK Buffer directly to the PK vial based on the chart below. Write the date of assembly on the vial. Mix well by gently shaking the vial. Store the PK solution at -20°C when not in use.

	<b>50 Prep Kit Part # A33341</b>	<b>96 Prep Kit Part # A33342</b>	<b>384 Prep Kit Part # A33343</b>
<b>Volume of PK Buffer to Add</b>	1.2 mL	2.3 mL	2.3 mL/ vial

- **Wash Buffer:** Add 100% Isopropanol to the Wash Buffer according to the chart below. Write the date of assembly on the label, then mix by inverting the bottle. Store the solution at room temperature.

	<b>50 Prep Kit</b>	<b>96 Prep Kit</b>	<b>384 Prep Kit</b>
<b>Volume of 100% Isopropanol</b>	15 mL	28 mL	112 mL

**For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186**

**1. Transfer FFPE tissue sections (up to 5 sections of 10 µm each) into a 1.7mL microcentrifuge tube.**

Agencourt recommends performing the upfront deparaffinization and digestions steps in tubes for ease in handling sections. Once completed, the magnetic extraction can take place in tubes or 96 well plates.

The optimal amount of starting material should be between 1-5, 10µm slices of tissue. For large samples, trim away excess paraffin around tissue. If sections are attached to glass slides, wet the sections with 20 µL of Lysis buffer *then* remove sections using a clean single-edge razor blade.

**2. Add 200 µL of Lysis buffer to each sample. Use the pipette tip to push the sections down into buffer until they are completely submerged.**

**3. Incubate the tube in a 70°C to 72°C water bath for 60 minutes.**

This step breaks down paraffin and releases the tissue. Remove samples promptly as prolonged incubation at 70°C may damage RNA. Quick spin to collect condensation.

**4. Add 20 µL of PK solution (prepared in Step 2). Be sure to pierce through the paraffin layer at the top of the liquid to ensure the PK goes directly into the sample. Pipette mix three times with a mix volume of 200 µL.**

The PK solution has a tendency to sink to the bottom of the sample, mix thoroughly to ensure good incorporation.

**5. Incubate the tube in a water bath at 55°C for 60 minutes.**

This step digests the tissue and inactivates nucleases. The digestion can be extended overnight. Quick spin to collect condensation.

**6. Cool the tube on ice for 2 minutes to solidify any excess paraffin.**

**7. Transfer 200 µL of lysate to a new 96 well plate or 1.7 mL tube for nucleic acid extraction. Avoid any solidified paraffin on the side walls.**

The transferred Agencourt FormaPure lysate can be stored at -20°C overnight for ease of processing.

**8. Add 150 µL of Bind 1 Buffer to each sample. Pipette mix 5 times with a mix volume of 300 µL.**

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- 9. Prepare Bind 2/Isopropanol solution: Shake or vortex Bind 2 Buffer vigorously to resuspend magnetic particles. For each individual isolation, combine 20 µL Bind 2 with 300 µL of 100% isopropanol in an RNase Free tube of suitable size. Mix well.**

Example: For 10 isolations, combine 200 µL of Bind 2 with 3 mL of 100% isopropanol in a 15 mL conical tube. Unused Bind 2/Isopropanol solution should be discarded.

- 10. Add 320 µL of Bind 2/Isopropanol solution (prepared in Step 9) to each sample. Pipette mix 5 times with a mix volume of 500 µL.**

- 11. Incubate in 55°C water bath for 5 minutes.**

Keep the incubator cover open since the plate is not sealed.

- 12. Move the plate/tube onto the Agencourt Supermagnet plate (or SPRISstand for tubes) and separate for 5 minutes. Slowly aspirate and discard the cleared supernatant.**

Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

- 13. Off the magnet, add 300 µL of Wash buffer (prepared in Step 2). Pipette mix 5 times to resuspend the beads, then incubate for 1 minute off the magnet.**

- 14. Separate on the magnet for 3 minutes. Carefully aspirate and discard the cleared supernatant.**

If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

- 15. Off the magnet, add 750 µL of 70% ethanol. Pipette mix 5 times with a volume of 500 µL to resuspend the beads.**

- 16. Separate on the magnet for 5 minutes. Carefully aspirate and discard the cleared supernatant.**

If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

- 17. Off the magnet, add 500 µL of 90% isopropanol. Pipette mix 5 times with a volume of 400 µL to resuspend the beads. Incubate in a 70°C water bath for 3 minutes.**

Keep the incubator cover open since the plate is not sealed.

- 18. Separate on the magnet for 3 minutes. Aspirate and discard the cleared supernatant.**

- 19. Repeat steps 17 and 18 for a total of 2 heated isopropanol washes.**

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**20. Off the magnet, add 750  $\mu$ L of 70% ethanol. Pipette mix 5 times with a volume of 500  $\mu$ L to resuspend the beads.**

**21. Separate on the magnet for 5 minutes, then carefully aspirate and discard the cleared supernatant.**

**22. Let the beads air dry for 10 minutes.**

The plate/tube should air-dry until the last visible traces of ethanol evaporate. Over-drying the sample may result in a lower recovery.

**23. Off the magnet, add 80  $\mu$ L of nuclease-free H<sub>2</sub>O. Resuspend the beads by pipette mixing 5 times. Incubate the plate/tube at 65°C to 70°C for 30 seconds.**

Keep the incubator cover open since the plate is not sealed.

The elution volume should be at least 40  $\mu$ L to ensure complete elution. Samples that produce higher yields require larger elution volumes due to potential bead carryover during the final transfer. Optimal elution volumes may need to be determined experimentally.

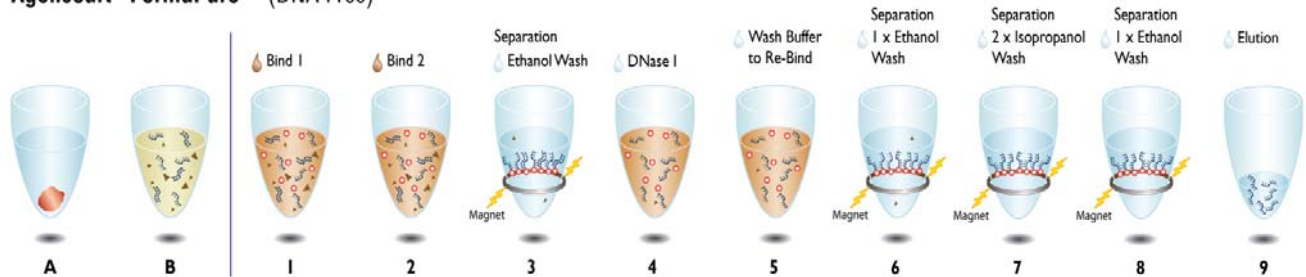
**24. Separate on the magnet for 1 minute, then transfer the eluant to a suitable 96 well plate or a fresh tube for storage.**

Wait for the solution to clear before transferring sample. When not in use, store the samples at -20°C.

**For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186**

**RNA Only - Process Overview**

**Agencourt® FormaPure™ (DNA Free)**



MANUAL

AUTOMATABLE

The Agencourt FormaPure procedure for the extraction of total RNA, is performed in the following stages:

**A & B.** Digestion of tissue and decrosslinking of nucleic acids

1. Addition of Bind 1 Buffer to lysate
2. Addition of Bind 2 Buffer to lysate
3. Separation of beads and washing of captured products with 70% ethanol
4. DNase I digestion
5. Addition of Wash Buffer to Rebind the RNA
6. Washing with 70% ethanol
7. Washing with 90% isopropanol
8. Washing with 70% ethanol
9. Elution of total RNA from the magnetic particles

**For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186**

**RNA Only - Materials Supplied by the User**

**Consumables and Hardware:**

- Agencourt Supermagnet Ring Magnetic Plate (Agencourt product # 000322; <http://www.agencourt.com>) or SPRISand for 1.7 mL tubes ( Agencourt product # 001139)
- Microtome for tissue sectioning
- **For Tube Format:**
  - 1.7 mL microcentrifuge tubes (ABGene Cat # T6050G; <http://www.abgene.com>)
- **For Plate Format:**
  - **For Manual Digestion/Processing 1.2 mL plates** (ABGene Cat # AB1127; <http://www.abgene.com>)
  - Adhesive plate film (ABGene Cat # AB-0558)
- 70-72°C and 55°C water bath
- **For RNA Only:**
  - 37°C water bath for DNase incubation.

**Reagents:**

- 100% Isopropanol, ultra pure (American Bioanalytical product # AB-07015; <http://www.americanbio.com/>)
- 90% Isopropanol made from ultra pure isopropanol and nuclease free water
- Fresh 70% ethanol made with nuclease free water (*Note: 70% ethanol is hygroscopic. Fresh 70% ethanol should be prepared for optimal results*) (American Bioanalytical product # AB-00138; <http://www.americanbio.com/>)
- **DNase I (RNase-Free) & DNase I Buffer** (2 U/μL); Ambion #AM2224 (contains both components), <http://www.ambion.com>
  - **OR DNase I (RNase-free)** (2 U/μL); Ambion #AM2222 **AND DNase I 10X Buffer** Ambion #AM8107G, <http://www.ambion.com>
- Reagent grade water, nuclease-free (Ambion product # 9932; <http://www.ambion.com>)

**RNA Only Extraction Using Agencourt FormaPure 96-well & Tube Protocol**

If you have already made the following preparations during a previous experiment, please start at Step 1.

**Before you start**

- **Preheat water baths to 72°C and 55°C.**
- **Ensure that Agencourt FormaPure Proteinase K and Wash Buffer have been assembled according to the following instructions:**

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- **Proteinase K (40 mg/mL):** Add PK Buffer directly to the PK vial based on the chart below. Write the date of assembly on the vial. Mix well by gently shaking the vial. Store the PK solution at -20°C when not in use.

	50 Prep Kit Part # A33341	96 Prep Kit Part # A33342	384 Prep Kit Part # A33343
<b>Volume of PK Buffer to Add</b>	1.2 mL	2.3 mL	2.3 mL/ vial

- **Wash Buffer:** Add 100% Isopropanol to the Wash Buffer according to the chart below. Write the date of assembly on the label, then mix by inverting the bottle. Store the solution at room temperature.

	50 Prep Kit	96 Prep Kit	384 Prep Kit
<b>Volume of 100% Isopropanol</b>	15 mL	28 mL	112 mL

**1. Transfer FFPE tissue sections (up to 5 sections of 10 µm each) into a 1.7 mL microcentrifuge tube.**

Agencourt recommends performing the upfront deparaffinization and digestions steps in tubes for ease in handling sections. Once completed, the magnetic extraction can take place in tubes or 96 well plates.

The optimal amount of starting material should be between 1-5, 10 µm slices of tissue. For large samples, trim away excess paraffin around tissue. If sections are attached to glass slides, wet the sections with 20 µL of Lysis buffer *then* remove sections using a clean single-edge razor blade.

**2. Add 200 µL of Lysis buffer to each sample. Use the pipette tip to push the sections down into buffer until they are completely submerged.**

**3. Incubate the tube in a 70°C to 72°C water bath for 60 minutes.**

This step breaks down paraffin and releases the tissue. Remove samples promptly as prolonged incubation at 70°C may damage RNA. Quick spin to collect condensation.

**4. Add 20 µL of PK solution (prepared in Step 2). Be sure to pierce through the paraffin layer at the top of the liquid to ensure the PK goes directly into the sample. Pipette mix three times with a mix volume of 200 µL.**

The PK solution has a tendency to sink to the bottom of the sample, mix thoroughly to ensure good incorporation.

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**5. Incubate the tube in a water bath at 55°C for 60 minutes.**

This step digests the tissue and inactivates nucleases. The digestion can be extended overnight. Quick spin to collect condensation.

**6. Cool the tube on ice for 2 minutes to solidify any excess paraffin.**

**7. Transfer 200 µL of lysate to a new 96 well plate or 1.7 mL tube for nucleic acid extraction. Avoid any solidified paraffin on the side walls.**

The transferred Agencourt FormaPure lysate can be stored at -20°C overnight for ease of processing.

**8. Add 150 µL of Bind 1 Buffer to each sample. Pipette mix 5 times with a mix volume of 300 µL.**

**9. Prepare Bind 2/Isopropanol solution: Shake or vortex Bind 2 Buffer vigorously to resuspend magnetic particles. For each individual isolation, combine 20 µL Bind 2 with 300 µL of 100% isopropanol in an RNase Free tube of suitable size. Mix well.**

Example: For 10 isolations, combine 200 µL of Bind 2 with 3 mL of 100% isopropanol in a 15 mL conical tube. Unused Bind 2/Isopropanol solution should be discarded.

**10. Add 320 µL of Bind 2/Isopropanol solution (prepared in Step 9) to each sample. Pipette mix 5 times with a mix volume of 500 µL.**

**11. Incubate in 55°C water bath for 5 minutes.**

Keep the incubator cover open since the plate is not sealed.

**12. Move the plate/tube onto the Agencourt Supermagnet plate (or SPRISstand for tubes) and separate for 5 minutes. Slowly aspirate and discard the cleared supernatant.**

Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

**13. Off the magnet, add 750 µL of 70% ethanol. Pipette mix 5 times with a volume of 500 µL to resuspend the beads.**

**14. Separate on the magnet for 5 minutes. Carefully aspirate and discard the cleared supernatant.**

If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

**15. Let the beads air dry for 5 minutes.**

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The plate/ tube should air-dry until the last visible traces of ethanol evaporate.

**16. Prepare fresh DNase solution and discard any unused solution.**

Combine 80 µL nuclease free water, 10 µL 10X DNase buffer and 10 µL of DNase I.

**17. Add 100 µL of DNase solution with the plate OFF the magnet. Pipette mix 5 times to resuspend the beads in the DNase solution.**

**18. Incubate plate/tube in a 37°C water bath for 15 minutes to facilitate digestion of DNA.**

Keep the incubator cover open since the plate is not sealed.

**19. DO NOT REMOVE THE DNase SOLUTION. Add 550 µL of Wash Buffer and pipette mix 5 times with a volume of 400 µL. Incubate at room temperature for 5 minutes.**

**20. Place plate/tube onto the magnet and separate for 10 minutes. Slowly aspirate the cleared solution from the plate/ tube and discard.**

This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

**21. Off the magnet, add 750 µL of 70% ethanol. Pipette mix 5 times with a volume of 500 µL to resuspend the beads.**

**22. Separate on the magnet for 5 minutes. Carefully aspirate and discard the cleared supernatant.**

If beads are drawn out, leave a few microliters of supernatant behind to avoid bead carryover.

**23. Off the magnet, add 500 µL of 90% isopropanol. Pipette mix 5 times with a volume of 400 µL to resuspend the beads. Incubate in a 70°C water bath for 3 minutes.**

Keep the incubator cover open since the plate is not sealed.

**24. Separate on the magnet for 3 minutes. Aspirate and discard the cleared supernatant.**

**25. Repeat steps 23 and 24 for a total of 2 heated isopropanol washes.**

**26. Off the magnet, add 750 µL of 70% ethanol. Pipette mix 5 times with a volume of 500 µL to resuspend the beads.**

**27. Separate on the magnet for 5 minutes, then carefully aspirate and discard the cleared supernatant.**

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**28. Let the beads air dry for 10 minutes.**

The plate/tube should air-dry until the last visible traces of ethanol evaporate. Over-drying the sample may result in a lower recovery.

**29. Off the magnet, add 80 µL of nuclease-free H<sub>2</sub>O. Resuspend the beads by pipette mixing 5 times. Incubate the plate/tube at 65°C to 70°C for 30 seconds.**

Keep the incubator cover open since the plate is not sealed.

The elution volume should be at least 40 µL to ensure complete elution. Samples that produce higher yields require larger elution volumes due to potential bead carryover during the final transfer. Optimal elution volumes may need to be determined experimentally.

**30. Separate on the magnet for 1 minute, then transfer the eluant to a suitable 96 well plate or a fresh tube for storage.**

Wait for the solution to clear before transferring sample. When not in use, store the samples at -20°C.

**LIMITED USE LABEL LICENSE**

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