app*GENE* Genomic DNA Kit Manual

Fast, Efficient, and Versatile

Cat No: ARN020 / 21
Ver1.0

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For the Life Scientist

DESCRIPTION

The **app*GENE* Genomic DNA kit** is highly versatile for rapidly isolating high quality genomic, mitochondrial, bacterial, parasite or viral DNA from multiple sources: tissues, physiological fluids, blood, swabs, semen, hair, rodent tails, insects, bacteria, yeast and eukaryotic cultured cells. Multiple samples of up to 1 x 10^7 eukaryotic cells or up to 30 mg of tissue can be processed in parallel in under 25 minutes. Purified genomic DNA can be used in many downstream applications such as PCR, qPCR, Southern blotting, genotyping DNA sequencing, restriction digests and ligations. The product is intended for research use only.

KIT COMPONENTS

Product Codes	**ARN020**	**ARN021**
Pack size	50 preps	250 preps
AGL buffer (Lysis buffer)	19ml	94ml
Proteinase K (lyophilized) ⊕	1 tube	5 tubes
Proteinase buffer	1.4ml	7ml
RNase A (lyophilized) ϕ	1 tube	5 tubes
RNase A buffer	220µl	1.1ml
AGB buffer (conc.)* (Binding Buffer) (volume of 96-100% ethanol to add)	10ml (15ml)	44ml (66ml)
AGW1 buffer (conc.)* (Wash Buffer 1) (volume of 96-100% ethanol to add)	17ml (17ml)	82ml (82ml)
AGW2 buffer (conc.)* (Wash Buffer 2) (volume of 96-100% ethanol to add)	9ml (21ml)	41ml (96ml)
Elution buffer	10ml	5x 10ml
app*GENE* DNA spin columns	50 columns	5 x 50 columns
Collection tubes (2ml)	50 tubes	5x 50 tubes
ARB Buffer (Blood Lysis Buffer)	50ml	250ml
AYS Buffer (Yeast Lysis Buffer)	10ml	50ml

BEFORE STARTING:

1. If processing the following sample types, ensure you have available the following reagents:

Sample Type	Reagent needed
Yeast	Lyticase
Staphylococcus species	Lysostaphin (400 U/ml) and 1x TE buffer
Enterococcus species	Lysozyme (100mg/ml) and 1x TE buffer
Cell cultures	1X PBS buffer
Formalin preserved tissues	1X PBS buffer
Physiological fluids	1X PBS buffer
Hair	1M DTT
Semen	1M DTT
Paraffin embedded tissues	Xylene
Sputum	Bromhexine or acetylcysteine

2. Prepare 1x PBS buffer by dissolving 8g NaCl, 0.2g KCl, 1.44g Na_2HPO_4 and 0.24g KH_2PO_4 in 800 ml H_2O. Set the pH to 7.4 with HCl. Fill up to 1000 ml and autoclave. Store at 4°C.
3. Prepare 1M DTT by dissolving 1.54 g DTT in 10 ml H_2O. Aliquot and store at -20°C.
4. Prepare 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Autoclave and store at 4°C.
5. Mix each buffer provided in the kit well, but do not mix the AGL buffer vigorously as it may foam due to its non-ionic detergent content. (To prevent foaming, centrifuge the buffer at 11,000 x g for 60 sec).
6. Examine the buffers provided in the kit. If sedimentation has occurred, incubate the RNase A or proteinase buffers at 50°C, and the AGB, AGW1 & AGW2 buffers at 37°C, mixing occasionally until the sediment has dissolved. Cool to room temperature.
7. $^{\oplus}$ - Prior to the first use, add 1,4 ml proteinase buffer per tube containing lyophilised proteinase K. Store at -20^{0}C.
8. $^{\phi}$ - Prior to the first use, add 220µl RNase A buffer to a tube containing the lyophilised RNase A. Store at 4^{0}C or long term at -20^{0}C.
9. * - Add appropriate amount of 96-100% ethanol to the AGB, AGW1 & AGW2 buffers; for details, see the instructions on the bottle label and in the table above. Marking the bottle after adding the alcohol is recommended.
10. Heat dry block heaters (or water baths) to 30°C or 37°C and 55°C depending on sample type being processed.

11. Unless otherwise stated, do all isolation steps at room temperature. It is advisable to do the entire procedure in a Class II Biological Safety cabinet when processing biological material in order to comply with safety requirements. We recommend the use of sterile filter pipette tips throughout.
12. Use a detergent-water solution to deal with spillage of guanidine salts from the buffers in the kits or with any contaminating liquids containing microorganisms.
13. If an RNA-free DNA sample is needed for downstream applications, add 4µl of RNase A solution to each homogenised sample and incubate at 37°C for 5 minutes, prior to following step 1 in the DNA protocol below.
14. Read the entire protocol bearing in mind the Considerations section to read tips about how to avoid issues like poor DNA integrity due to incorrect storage or the use of inappropriately treated consumables, inefficient elution due to lack of washing, and how to amend the elution volume depending on the concentration of DNA required.

STORAGE

Store the app*GENE* Genomic DNA kit at room temperature (18 - 25^{o}C). After reconstitution, proteinase K should be stored at -20^{0}C and RNase A can be stored short term at 4^{0}C or long term at - 20^{0}C. Avoid evaporation from buffer bottles by ensuring they are tightly closed before storing. Under proper storage the kit will remain stable for at least 12 months.

SAMPLE PREPARATION

1. TISSUE

1.1 Homogenisation via Mortar and Pestle

1. Put ≤ 30mg tissue (fresh or previously frozen in liquid nitrogen / dry ice) into a pre-chilled, sterile mortar. If processing ≤ 30mg of formalin-preserved tissue, wash three times using sterile pre-cooled 1x PBS buffer before homogenisation.
2. Using a chilled pestle, carefully, but firmly crush the tissue into smaller pieces and then, into a pulp / powder.
3. Transfer the pulp / powder thus obtained into a sterile DNase-free 2ml microcentrifuge tube containing 375µl AGL buffer and go to step 2 of the protocol. (After pulping, a thin, sticky layer may be formed, rather than a powder. If this occurs, add 375µl AGL buffer to the mortar and reconstitute the tissue by pipetting and then transfer the lysate into a sterile DNase-free 2ml microcentrifuge tube. Retrieve the tissue remains from the pestle as well).

1.2 Homogenisation via Mechanical Homogenizer

1. Place ≤ 30mg tissue (fresh or previously frozen in liquid nitrogen / dry ice) in a sterile DNase-free 2ml microcentrifuge tube. If processing ≤ 30mg of formalin-preserved tissue, wash three times using sterile pre-cooled 1x PBS buffer before homogenisation.
2. Add 100µl AGL buffer and carefully homogenize with a sterile homogenizer tip.
3. After homogenization, retrieve the tissue remains from the knife tip by washing it with 275µl AGL buffer.
4. Combine the fractions thus obtained and transfer the entire volume to a new sterile, DNase-free 2ml microcentrifuge tube.
5. Go to step 2 of the below protocol.

1.3 Homogenisation of Paraffin-embedded Tissue

1. Cut out ≤ 30mg of paraffin-embedded tissue out of a paraffin block and place it into a sterile, DNase-free 2ml microcentrifuge tube.
2. Add 1ml xylene to the slice of tissue and vortex for 30 sec in a fume hood, as xylene is toxic, an irritant and inflammable.
3. Centrifuge for 5 min at 15,000 x g and then carefully remove the supernatant by pipetting.
4. Repeat steps 2-3.
5. Add 1ml 96-100% ethanol and mix by vortexing for 15 sec.
6. Centrifuge for 2mins at 15 000 x g and then carefully remove the supernatant by pipetting.
7. Repeat steps 5-6.
8. Dry the pellet at 50°C for 5-20mins.
9. Add 375µl AGL buffer and vortex for 20 sec to mix.
10. Go to step 2 of the below protocol.

2. CELL CULTURES

1. Thaw 10^3-10^7 cell suspension or adherent cells at 37°C, (cells should be either fresh or frozen at -80^0C / -196^0C).
2. Suspend the cells in growth medium or 1x PBS buffer in a 15ml sterile centrifuge tube or a 1.5-2ml sterile, DNase-free microcentrifuge tube and centrifuge them at 3000 x g.
3. If a compact cell pellet is not formed, wash the cells twice with 1ml cold PBS buffer.
4. Add 375µl AGL buffer. Mix thoroughly by vortexing for 30 sec and subsequent pipetting. In some cases when cells tend to form syncytia (myoblasts) or tight connections (epithelial cells) or they are high in number (approx. 10^7 cells), it may be difficult to re-suspend them in AGL buffer. In such cases, pipette carefully, using a ≥1000µl pipette tip or a sterile syringe. Do not use filter tips.
5. Transfer everything to a new 2ml sterile, DNase-free microcentrifuge tube.
6. Go to step 2 of the below protocol.

3. GRAM-NEGATIVE BACTERIA

3.1 Processing 3ml of Bacterial Broth

The bacterial volume range that can be processed per spin column is 0.2-3ml. If processing 3ml, first transfer 1.5ml to a sterile 1.5–2ml DNase-free microcentrifuge tube, spin down the cells at 4000 x g, remove the supernatant, and add an extra 1.5ml of the culture to the top of the cell pellet, spin it again and re-suspend in 375µl of AGL buffer. Go to step 2 of the below protocol.

3.2 Processing >10^9 Cells

The bacterial cell density that can be processed is ≤ 10^9 cells. If processing more than 10^9 cells, spin down the cells at 4000 x g, remove the supernatant and re-suspend in 450µl of AGL buffer. Add 15µl of proteinase K and incubate at 55°C for 10 min, followed by 6µl of RNase A and incubation at 37°C for 5min. Go to step 4 of the below protocol.

3.3 Processing Frozen Bacterial Cells

Immediately re-suspend the frozen cell pellet in 300µl of AGL buffer and do not allow the cell pellet to thaw. Go to step 2 of the below protocol.

3.4 Processing Bacteria Colonies from Plates

Take a sufficient amount of the bacteria using a sterile loop and re-suspend it into 300µl of AGL buffer to a sterile 1.5 ml DNase-free microcentrifuge tube. Go to step 2 of the below protocol.

4. GRAM-POSITIVE BACTERIA

4.1 Processing *Staphylococci* Strains

1. Spin down 1.5ml of bacterial culture at 4000 x g, remove the supernatant and re-suspend the cell pellet thoroughly into 200µl of 1x TE buffer.
2. Add 30µl lysostaphin (400 U/ml) and 4µl RNase A. Mix well by pipetting and incubate at 37°C for 20–60 min until the sample is lysed. (When isolating from coagulase-negative strains, use 50µl of lysostaphin and incubate 1 hr at 37°C).
3. Add 300µl AGL buffer and 17µl proteinase K. Mix thoroughly and incubate at 55°C for 10 min.
4. Go to step 4 of the below protocol.

4.2 Processing *Enterococci* Strains

1. Spin down 1.5ml of bacterial culture at 4000 x g, remove the supernatant and re-suspend the cell pellet thoroughly into 200µl of 1x TE buffer.
2. Add 40µl lysozyme (100 mg/ml) and 4µl RNase A. Mix well by pipetting and incubate at 37°C for 40–60 min until the sample is lysed.
3. Add 300µl AGL buffer and 17µl proteinase K. Mix thoroughly and incubate at 55°C for 10 min.
4. Go to step 4 of the below protocol.

5. YEAST

1. Pellet 0.2-3ml yeast (≤ 10^8 cells) from broth, plate or frozen cell pellet, at 4000 x g.
2. Remove the supernatant and re-suspend the cell pellet thoroughly into 200µl of AYS buffer.
3. Add 50 - 200 units of lyticase and mix by vortexing.
4. Incubate at 30°C for 30 min and occasionally invert the tube to mix until the sample is lysed.
5. Centrifuge at 1000 x g for 10min.
6. Remove the supernatant using a pipette tip – be careful not to disturb the spheroplast pellet.
7. Go to step 2 of the below protocol.

6. PHYSIOLOGICAL FLUID

6.1 Urine, Cerebrospinal / Peritoneal/Pleural fluids

1. Centrifuge ≤ 5ml of fluid at 500 x g for 5 mins and discard the supernatant.
2. Wash the cell pellet with 1ml 1x PBS buffer by pipetting and centrifuge for 1 min at 3000 x g.
3. Add 375µl AGL buffer. Mix thoroughly by vortexing for 30 sec.
4. Go to step 2 of the below protocol.

6.2 Sputum

1. Add 0.5-10% bromhexine / acetylcysteine (mucolytic agents) at a 1:1 ratio to ≤ 5ml of sputum.
2. Centrifuge at 3000 x g for 1 min and discard the supernatant.
3. Wash the cell pellet with 1ml 1x PBS buffer by pipetting and centrifuge for 1 min at 3000 x g.
4. Add 375µl AGL buffer. Mix thoroughly and re-suspend the cell pellet by vortexing for 30 sec.
5. Go to step 2 of the below protocol.

7. BLOOD

1. Pipette 50 -1000µl of blood into sterile 1.5-2ml DNase-free microcentrifuge tube and an equal volume of ARB lysis buffer. If <200µl of blood is being processed, make the sample up to a final volume of 200µl with elution buffer, and then add 200µl of ABC lysis buffer.
2. Mix well by inversion until a clear red solution is obtained.
3. Centrifuge at 8,600 x g for 4 mins and discard the supernatant. (Do not spin at a higher speed as this may prevent white blood cells from being adequately suspended in lysis buffer downstream).
4. Remove the supernatant using a pipette tip – be careful not to disturb the white blood cell pellet.
5. Add 375µl AGL buffer. Mix thoroughly and re-suspend the cell pellet by vortexing for 20 sec.
6. Add 10µl proteinase K and mix by inversion and incubate at 55°C for 10 min.
7. Go to step 3 of the below protocol.

8. SEMEN

1. Pipette ≤ 150µl of semen into sterile 1.5-2ml DNase-free microcentrifuge tube. (If <150µl of semen is being processed, make the sample up to a final volume of 150µl with elution buffer).
2. Add 375µl AGL buffer, 10µl proteinase K and 20µl 1M DTT and vortex for 3 sec.
3. Incubate at 55°C for 30 min. At regular intervals during the incubation, mix the sample by inversion, or use a thermomixer.
4. Go to step 3 of the below protocol.

9. SWABS

1. Place the swab into a sterile 1.5-2ml DNase-free microcentrifuge tube and cut off the excess from the end of the shaft so that the lid can be closed.
2. Add 375µl AGL buffer and 10µl proteinase K and vortex for 3 sec.
3. Incubate at 55°C for 30 min. At regular intervals during the incubation, mix the sample by inversion, or use a thermomixer.
4. After 30mins, press the swab against the side of the tube to squeeze out as much of the lysate as possible and discard the swab.
5. Go to step 3 of the below protocol.

10. HAIR

1. Take 10-30mg of hair preferably with hair roots attached (100 – 120 strands) or (≤ 30mg hair roots).
2. Cut off the hair roots and transfer them to a sterile 1.5-2ml DNase-free microcentrifuge tube. If there are no hair roots, cut the hair shafts into 3mm pieces and transfer them to a sterile 1.5-2ml DNase-free microcentrifuge tube. (Hair roots contain living cells whereas the rest of the hair carries only traces of degraded genomic or mitochondrial DNA, so downstream applications need to take this into account and design primers to amplify up products ≤200bp).
3. Add 375µl AGL buffer, 25µl proteinase K and 40µl 1M DTT and vortex for 30 sec.
4. Incubate at 55°C overnight or for a minimum of 6 hrs. Vortex for 1-2mins at regular intervals during the incubation or use a thermomixer.
5. Go to step 3 of the below protocol.

11. RODENT TAILS

1. Cut ≤30mg of rat or mouse tail into smaller fragments, and homogenize them manually using a pestle and mortar or via mechanical homogenizer.
2. Transfer them to a sterile 1.5-2ml DNase-free microcentrifuge tube and add 375µl AGL buffer, and vortex vigorously for 20 sec. Add 25µl proteinase K and 4µl RNase A and incubate at 37°C for 5 mins and then at 55°C for 2-3hrs for well homogenised samples or for a minimum of 5-16hrs for small fragments. Vortex vigorously for 20sec every 1-2hrs during the incubation or use a thermomixer.
3. Go to step 4 of the below protocol.

12. INSECTS

1. Wash ≤30mg of insects at various stages of life (fresh, frozen or preserved in formalin/ethanol) with 1x PBS / distilled water twice and centrifuge at 500 x g for 1 min.
2. Cut into smaller fragments, and homogenize them manually using a pestle and mortar until well powdered.
3. Transfer the powder to a sterile 1.5-2ml DNase-free microcentrifuge tube and add 375µl AGL buffer, and vortex vigorously for 1 min.
4. Add 25µl proteinase K and 4µl RNase A and incubate at 37°C for 5 mins and then at 55°C for 2-3hrs for well homogenised samples or for a minimum of 5-16hrs for small fragments.
5. Vortex vigorously for 20sec every 1-2hrs during the incubation or use a thermomixer.
6. Go to step 4 of the below protocol.

DNA EXTRACTION PROTOCOL

1. Having added the appropriate volume of AGL buffer based on the sample type (see above sections), then vortex for 20 secs.

2. Add 25µl proteinase K and incubate at 55°C for 2-3hrs for well homogenised samples or for a minimum of 5-16hrs for small fragments until the material has been completely digested into a viscous liquid. Vigorously mix the sample throughout the incubation for 20 sec every 30-60 mins (or use a thermomixer).

3. Digest any contaminating RNA in the sample by adding 4µl RNase A and incubating at 37°C for 5 mins.

4. Add 400µl AGB buffer and mix thoroughly for 10 secs.

5. Centrifuge for 2 mins at 21,000 x g.

6. Transfer the supernatant into an app*GENE* DNA spin column placed in a collection tube. (Do not transfer any tissue remains into the column).

7. Centrifuge for 1 min at 15,000 x g. (Ensure the inside of the spin column is dry after the spin. If not completely dry, re-spin for 2 mins at 21,000 x g.

8. Discard the flow-through and place the app*GENE* DNA spin column in a new collection tube.

9. Add 600µl AGW1 buffer and centrifuge for 30 sec at 15,000 x g.

10. Discard the flow-through and re-use the collection tube.

11. Add 500µl AGW2 Buffer and centrifuge for 30 sec at 15,000 x g.

12. Discard the flow-through and re-use the collection tube.

13. Centrifuge for 2 mins at 21,000 x g (read Considerations, Section 1.3 on washing).

14. Discard the collection tube and flow-through and carefully transfer the app*GENE* DNA spin column to a sterile, 1.5ml sterile, DNase-free microcentrifuge (read Considerations, Section 1.4 on elution).

15. Add 50-200µl elution buffer precisely, onto the centre of the app*GENE* DNA spin column membrane.

16. Centrifuge at 15,000 x g for 1 min.

17. Remove the app*GENE* DNA spin column and place the tube with the eluted DNA in a freezing rack. The isolated DNA is ready for use in downstream applications or for short term storage at 4°C or long term storage at -20°C.

CONSIDERATIONS

1.1 Tissue Storage Prior to DNA Extraction

Proper sampling and storing of the biological material prior to DNA isolation is crucial to obtaining a high purity DNA sample. After sampling, the biological material should either be preserved by deep freezing at -80°C or in liquid nitrogen or stored in RNase A inactivating buffers at -20°C. Most tissues must be preserved within 30 minutes of sampling. Tissues rich in enzymes (pancreas, liver) must be preserved immediately. When isolating from cell cultures, the best results are achieved with fresh material. If storage is unavoidable, discard the supernatant after centrifugation and freeze the cell pellet at -80°C or in liquid nitrogen.

When isolating from more than the recommended quantity of starting material (>30 mg, $>10^7$ cells), divide the material into several isolations so that each 30mg (or 10^7 cells) of sample material is isolated with a separate buffer and spin column set. If this quantity is exceeded, the homogenizing column may become clogged and/or the isolated DNA may be of low purity.

1.2 DNases

In order to avoid the degrading effect of DNases on DNA, the following recommendations should be followed:

- Use disposable latex, vinyl or nitrile gloves at all times when working with the DNA. Do not touch any items not designed specifically for DNA work.
- Disposable plastic ware (tips, tubes) should be DNase-free or autoclaved at 134°C for 18-20 minutes.
- Re-useable plastic ware, glass and porcelain should be soaked overnight in 0.1 N NaOH/0.1% DEPC water (or RNase-free water) and then washed with 0.1% DEPC water (or RNase A-free water). When applicable, glass and porcelain (mortars) should be parched at 150-140°C for 2-4 hrs and cooled to room temperature.

1.3 Washing

The wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from the app*GENE* DNA spin column prior to elution.

1.4 Elution

The optimal volume of the elution buffer used should be chosen in line with the amount of the sample material and the final DNA concentration expected.

Sample Type	Amount	Volume of Elution buffer	Comment
Tissue	2 – 10mg	100–200µl	
Cells	10^4 cells	100–200µl	
Tissue	10 – 30mg	200µl	
Cells	10^4 – 10^7 cells	200µl	
Swab	single buccal swab	50–100µl	
Semen	150µl	50–100µl	
Bacteria	10^9 cells	50–100µl	use 200µl for > 10^9 cells
Yeast	10^8 cells	50–100µl	use 200µl for > 10^9 cells
Blood	100 – 500µl containing 3x 10^6 – 1x 10^7 cells/ml	50–100µl	
Blood	500 – 1000µl containing 3x 10^6 – 1x 10^7 cells/ml	200µl	

If a high DNA concentration is desired, the elution volume may be reduced. However, it should be noted that this may reduce the efficiency of the DNA retrieval. It is essential to apply the elution buffer precisely to the centre of the membrane. When more sample material is to be used for isolation (not recommended as the column can then easily become clogged), full DNA retrieval can be obtained by performing a second elution (50-200µl). For the second elution, repeat steps 15 to 17 of the above protocol, placing the app*GENE* DNA spin column in a new, sterile 1.5ml microcentrifuge tube. The elution buffer does not contain EDTA, which may interfere with some enzymatic reactions. DNA can also be eluted from the spin column using nuclease-free water (pH 7.0 – 9.0) or 1x TE buffer.

1.5 RNA contamination

The app*GENE* Genomic DNA kit provides efficient digestion of the RNA prior to DNA purification. Most fresh or frozen tissue contains more RNA than DNA, especially metabolically active tissues like glands, nerve tissue and epithelium. RNA may interfere with some enzymatic reactions, but does not inhibit PCR. If an RNA-free DNA sample is desired, add 4µl of RNase A solution and incubate at 37°C for 5 minutes (step 3 of the above protocol).

1.6 Average DNA Yields from Different Sample Types

Sample Material	Amount	DNA Yield
Rat Liver	30mg	47 µg
Rat Skeletal Muscle	20mg	10.1 µg
Rat Heart	20mg	24.7 µg
Yellow adipose tissue	30mg	9.8 µg
Brown adipose tissue	30mg	23.3 µg
Rat kidney	20mg	36.7 µg
HT29 cell culture	1×10^5	10.3 µg
HCT116 cell culture	3×10^6	12.0 µg
Rat brain	20mg	1.88 µg
Insects	2.7mg	0.39 µg
Blood	200µl	3-10 µg
Yeast	1×10^8	1.5 µg
Swab	1 swab	3 µg
Semen	150µl	2-7 µg
Rodent tail	30mg	35 µg

TROUBLE SHOOTING / TECHNICAL SUPPORT

For troubleshooting please visit www.appletonwoods.co.uk/app*GENE*-Genomic-DNA-kit-troubleshooting.pdf for a trouble shooting guide.

NOTES

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ASSOCIATED PRODUCTS

Product	Pack Size	Product Code
app*GREEN* 1-Step Extreme Low ROX Kit	200 reactions	ARP742
app*GREEN* 1-Step Extreme High ROX Kit	200 reactions	ARP752
app*GREEN* 1-Step Opti Low ROX Kit	200 reactions	ARP792
app*GREEN* 1-Step Opti High ROX Kit	200 reactions	ARP802
app*TAQ* Polymerase	500 units	ARP001
app*TAQ* RedMix (2X)	200 reactions	ARP062
app*TAQ* Hot Start Polymerase	250 units	ARP021
app*HiFi* Polymerase	200 units	ARP041
appMEGA Polymerase	250 units	ARP031
Molecular biology grade water	100mL	BMW001

More pack sizes available at www.appletonwoods.co.uk

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