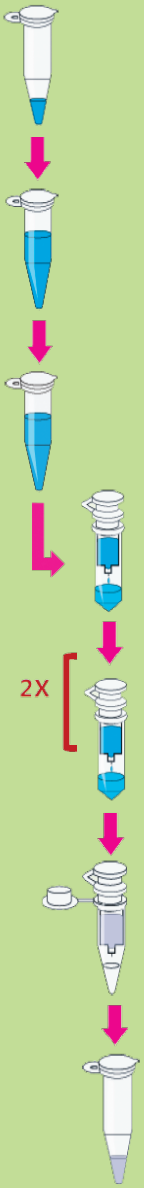
Quick Guide

MYgen Total RNA Prep Kit - Tissue Culture Cell Protocol

Products	Cat No.	Size
MYgen Total RNA Prep Kit (For Bacterium, Plant, AnimalTissue, Tissue cultured Cell)	MYR101-050	50 preps
	MYR101-100	100 preps

Kit Contents:
RB, Proteinase K
RW, RNase Free Water
HiSpin Capsule



1. Harvest Cells (< 1 x 10^7)
   Add 350 uL RB1 (+2-ME)
   Add 5 uL Proteinase K
   Vortex (30 sec - 10 mins)

2. Incubation (56^{o}C, 10 mins)
   Cfg (14,000 rpm, 3 mins)
   Transfer supernatant to new 1.5mL micro tube

3. Add 250 uL Ethanol (100%)
   Vortex (30 sec- 1 min)

4. Add lysate/ethanol mix to spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through

5. Add 500 uL RW into spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
   Repeat RW washing step again

6. Cfg (14,000 rpm, 3 mins, 4^{o}C)
   - remove final wash buffer residual
   Replace a new 1.5mL micro tube

7. Add 50-100 uL RNase free water
   Incubate mixture (RT, 1 min)
   Cfg (14,000 rpm, 1min)

RNA Elution
Store at -70^{o}C

Preparation:

- 2-Mercaptoehanol(2-ME) should be added to RB solution and the mixed RB/2-ME Solution should be stored at 4^{o}C. (Ratio of addition: 10 uL 2-ME : 1mL RB Solution)
- Add 100% ethanol to RW according to label before use.
- Add Deionized Water to lyophilised Proteinase K according to label and mix well. Store at -20^{o}C.

Protocol:

1. Vortex mixture of hasvest cells (< 1 x 10^7) + 350 uL RB(+2-ME) + 5 uL Proteinase K and incubation at 56^{o}C for 10 mins.

2. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C and transfer the supernatant into a new 1.5mL micro tube.

3. Add 250 uL Ethanol (100%) into lysate and vortex for 30 sec – 1 mins

4. Place the spin column into a 2 mL collection tube then
   - Add Lysate/Ethanol mix from Step 3 into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again

5. Add 500 uL RW (RNA washing solution) into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again
   - Repeat above steps for washing

6. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C to remove residual wash buffer
   - Place the spin column into a new 1.5 mL micro tube

7. Add 50 - 100 uL RNase free water
   - Incubate the mixture for 1 min at RT
   - Perform centrifugation at 14,000 rpm for 1 min
   - Remove the Spin column
   - Store at -70^{o}C

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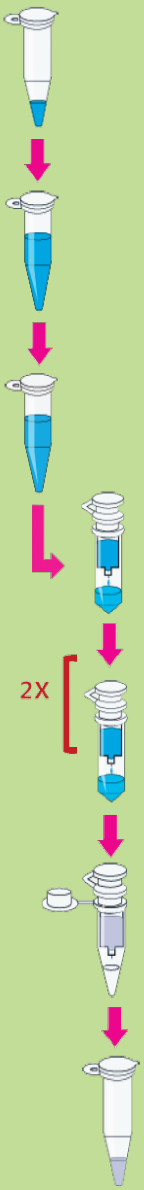
Quick Guide

MYgen Total RNA Prep Kit - Plant Tissue Protocol

Products	Cat No.	Size
MYgen Total RNA Prep Kit (For Bacterium, Plant, AnimalTissue, Tissue cultured Cell)	MYR101-050	50 preps
	MYR101-100	100 preps

Kit Contents:
RB, Proteinase K
RW, RNase Free Water
HiSpin Capsule



1. Plant tissue (< 100 mg)
   Add 350 uL RB1 (+2-ME)
   Vortex (30 sec - 1 min)
2. Incubation (60^{o}C, 3-10 mins)
   Cfg (14,000 rpm, 3 mins)
   Transfer supernatant to
   new 1.5mL micro tube
3. Add 250 uL Ethanol (100%)
   Vortex (30 sec- 1 min)
4. Add lysate/ethanol mix to spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
5. Add 500 uL RW into spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
   Repeat RW washing step again
6. Cfg (14,000 rpm, 3 mins, 4^{o}C)
   - remove final wash buffer residual
   Replace a new 1.5mL micro tube
7. Add 50-100 uL RNase free water
   Incubate mixture (RT, 1 min)
   Cfg (14,000 rpm, 1min)

   RNA Elution
   Store at -70^{o}C

Preparation:

- 2-Mercaptoehanol(2-ME) should be added to RB solution and the mixed RB/2-ME Solution should be stored at 4^{o}C. (Ratio of addition: 10 uL 2-ME : 1mL RB Solution)
- Add 100% ethanol to RW before use.
- Sample
  (It is recommended to start with no more than 100 mg plant tissue. Grind the fresh-freeze tissues with liquid nitrogen thoroughly. Keep the samples cool and perform the following steps promptly to minimize RNA degradation)

Protocol:

1. Vortex mixture sample (< 100 mg) + RB(+2-ME) 350 uL for 30 sec - 1 min and incubation at 60^{o}C for 3 mins (Note: Perform incubation at 60^{o}C for 5 - 10 mins in case of high starch or polyphenol / polysaccharide sample)

2. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C and transfer the supernatant into a new 1.5mL micro tube.

3. Add 250 uL Ethanol (100%) into lysate and vortex for 30 sec – 1 mins

4. Place the spin column into a 2 mL collection tube then
   - Add Lysate/Ethanol mix from Step 3 into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again

5. Add 500 uL RW (RNA washing solution) into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again
   - Repeat above steps for washing

6. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C to remove residual wash buffer
   - Place the spin column into a new 1.5 mL micro tube

7. Add 50 - 100 uL RNase free water
   - Incubate the mixture for 1 min at RT
   - Perform centrifugation at 14,000 rpm for 1 min
   - Remove the Spin column
   - Store at -70^{o}C

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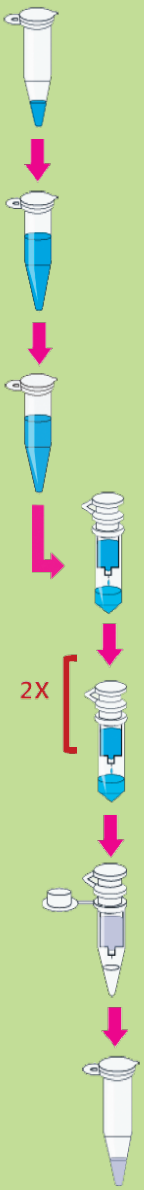
Quick Guide

MYgen Total RNA Prep Kit - Bacterium Protocol

Products	Cat No.	Size
MYgen Total RNA Prep Kit (For Bacterium, Plant, AnimalTissue, Tissue cultured Cell)	MYR101-050	50 preps
	MYR101-100	100 preps

Kit Contents:
RB, Proteinase K
RW, RNase Free Water
HiSpin Capsule



1. Harvest Bacteria (< 1 x 10^9)
   Add 100 uL TE
   Add 2 uL of Lysozyme
   Vortex (30 sec - 1 min)
   Incubation (RT, 3 - 10 mins)
2. Add 350 uL RB1 (+2-ME)
   Vortex (30 sec - 10 mins)
   Cfg (14,000 rpm, 3 mins)
   Transfer supernatant to new 1.5mL micro tube
3. Add 250 uL Ethanol (100%)
   Vortex (30 sec- 1 min)
4. Add lysate/ethanol mix to spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
5. Add 500 uL RW into spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
   Repeat RW washing step again
6. Cfg (14,000 rpm, 3 mins, 4^{o}C)
   - remove final wash buffer residual
   Replace a new 1.5mL micro tube
7. Add 50-100 uL RNase free water
   Incubate mixture (RT, 1 min)
   Cfg (14,000 rpm, 1min)

   RNA Elution
   Store at -70^{o}C

Preparation:

- 2-Mercaptoehanol(2-ME) should be added to RB solution and the mixed RB/2-ME Solution should be stored at 4^{o}C. (Ratio of addition: 10 uL 2-ME : 1mL RB Solution)
- Add 100% ethanol to RW according to label before use.
- Add Deionized Water to lyophilised Lysozyme according to label and mix well. Store at -20^{o}C.
- Sample
  i. Gram-negative bacteria: ~ 500 ug
  ii. Gram-positive bacteria: ~ 3 mg / mL

Protocol:

1. Vortex mixture of hasvest bacteria (< 1 x 10^9) + TE buffer 100 uL + Lysozyme 2 uL and incubate:
   i. Gram-negative bacteria: 3 - 5 mins, RT
   ii. Gram-positive bacteria: 5 - 10 mins, RT

2. Add in 350 uL RB(+2-ME) and vortex for 30 sec - 1 min
   - Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C and transfer the supernatant into a new 1.5mL micro tube.

3. Add 250 uL Ethanol (100%) into lysate and vortex for 30 sec – 1 mins

4. Place the spin column into a 2 mL collection tube then
   - Add Lysate/Ethanol mix from Step 3 into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again

5. Add 500 uL RW (RNA washing solution) into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again
   - Repeat above steps for washing

6. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C to remove residual wash buffer
   - Place the spin column into a new 1.5 mL micro tube

7. Add 50 - 100 uL RNase free water
   - Incubate the mixture for 1 min at RT
   - Perform centrifugation at 14,000 rpm for 1 min
   - Remove the Spin column
   - Store at -70^{o}C

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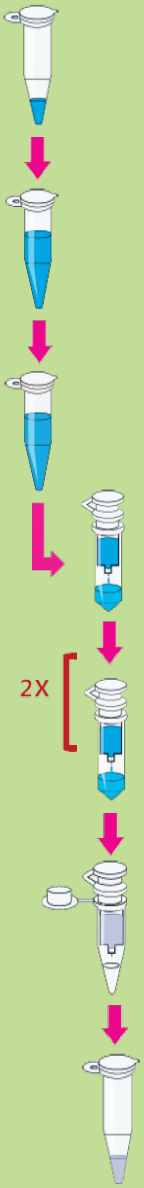
Quick Guide

MYgen Total RNA Prep Kit - Animal Tissue Protocol

Products	Cat No.	Size
MYgen Total RNA Prep Kit (For Bacterium, Plant, AnimalTissue, Tissue cultured Cell)	MYR101-050	50 preps
	MYR101-100	100 preps

Kit Contents:
RB, Proteinase K
RW, RNase Free Water
HiSpin Capsule



1. Animal tissue (< 30 mg)
   Add 350 uL RB1 (+2-ME)
   Add 5 uL of Proteinase K
   Vortex (30 sec - 1 min)
2. Incubation (56^{o}C, 10 mins)
   Cfg (14,000 rpm, 3 mins)
   Transfer supernatant to
   new 1.5mL micro tube
3. Add 250 uL Ethanol (100%)
   Vortex (30 sec- 1 min)
4. Add lysate/ethanol mix to spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
5. Add 500 uL RW into spin column
   Cfg (14,000 rpm, 30 sec, 4^{o}C)
   Discard flow-through
   Repeat RW washing step again
6. Cfg (14,000 rpm, 3 mins, 4^{o}C)
   - remove final wash buffer residual
   Replace a new 1.5mL micro tube
7. Add 50-100 uL RNase free water
   Incubate mixture (RT, 1 min)
   Cfg (14,000 rpm, 1min)

   RNA Elution
   Store at -70^{o}C

Preparation:

- 2-Mercaptoehanol(2-ME) should be added to RB solution and the mixed RB/2-ME Solution should be stored at 4^{o}C. (Ratio of addition: 10 uL 2-ME : 1mL RB Solution)
- Add 100% ethanol to RW according to label before use.
- Add Deionized Water to lyophilised Proteinase K according to label and mix well. Store at -20^{o}C.
- Sample
  (It is recommended to start with no more than 30 mg animal tissue. Grind the fresh-freeze tissues with liquid nitrogen thoroughly. Keep the samples cool and perform the following steps promptly to minimize RNA degradation)

Protocol:

1. Vortex mixture sample (< 30 mg) + RB(+2-ME) 350 uL + Pro-K (10 mg/ml) 5uL for 30 sec - 1 min and incubation at 56^{o}C for 10 mins

2. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C and transfer the supernatant into a new 1.5mL micro tube.

3. Add 250 uL Ethanol (100%) into lysate and vortex for 30 sec – 1 mins

4. Place the spin column into a 2 mL collection tube then
   - Add Lysate/Ethanol mix from Step 3 into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again

5. Add 500 uL RW (RNA washing solution) into spin column
   - Perform centrifugation at 14,000 rpm for 30 sec, 4^{o}C
   - Discard the flow-through and place the spin column into a 2 mL collection tube again
   - Repeat above steps for washing

6. Perform centrifugation at 14,000 rpm for 3 mins, 4^{o}C to remove residual wash buffer
   - Place the spin column into a new 1.5 mL micro tube

7. Add 50 - 100 uL RNase free water
   - Incubate the mixture for 1 min at RT
   - Perform centrifugation at 14,000 rpm for 1 min
   - Remove the Spin column
   - Store at -70^{o}C

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