



Instructions for use

ROTI®Prep gDNA Mini 2.0

1YTK

Kit for the isolation of genomic DNA from various starting materials such as bacteria, fungi, cell cultures or blood.

1. Introduction and product description

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation in approx. 8 minutes (excluding lysis)
- Yield up to 65 µg gDNA, depending on source material
- Average Ratio $A_{260}:A_{280}$: 1.8-2,0

ROTI®Prep gDNA Mini 2.0 has been developed for the isolation of genomic DNA (gDNA) from various sources. The kit is a further development of our proven ROTI®Prep genomic DNA (8472) and can now be used with significantly more source materials, such as blood, fungi, bacteria or spores.

The isolation is carried out through the proven and reliable spin column technique, which is very easy to handle and needs only few steps. The extraction is based on a new procedure and combines sample lysis with the subsequent binding of the DNA to the surface of a column membrane. After several washing and centrifugation steps, the DNA is eluted from the membrane using an elution buffer. The extraction procedure in this kit is optimised to achieve maximum yield. The thereby extracted DNA is free of RNA and proteins and can directly be stored or used for downstream applications.

Suitable source material:

- Microbial cell cultures (max. 5×10^{10})
- Cell cultures (max. 5×10^6)
- Rodent tail (0,5-1 cm)
- Buccal swabs
- Tissue samples (max. 20 mg)
- Blood (200-400 µl)

2. Product use and warranty

2.1 Intended Use

- For research use only.
- Please read the instructions for use carefully and follow the instructions accordingly. Reliability of results cannot be guaranteed if there are any deviations in the procedure.

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Literature Citation: When describing a procedure for publication using this product, please refer to it as *Carl Roth's ROTI®Prep gDNA Mini 2.0 Kit*

2.2 Safety Precautions

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.


Caution:

Lysis Buffer LSV   Warning H318-H302

Lysis Buffer LSN   Warning H318-H302

Binding Buffer BR   Danger H225-H319-H336

Binding Buffer BSN   Danger H225-H319-H336

Proteinase K*   Danger H315-H319-H334-H317-H335

Washing Solution WSO   Danger H225-H319-H336

MSDS: the appropriate MSDS can be downloaded from our website www.carlroth.com.

Attention: Do not add bleach or acidic components to the waste after sample preparation!

3. Materials provided in this kit and storage conditions

3.1. Included Kit components

Amount	Component	Storage
5 / 25 / 120 ml	Lysis Buffer LSV	RT
2 / 12 / 60 ml	Lysis Buffer LSN	RT
8 / 40 / 200 ml	Binding Buffer BR	RT
2 / 12 / 60 ml	Binding Buffer BSN	RT
For 2x 0.3/ 2x 1.5 / 6x 1.5 ml	Proteinase K*	+4 °C
60 / 300 / 2x 300 µl	RNase A (10 mg/ml)	-20 °C
5 / 25 / 120 ml	Washing Solution WSO	RT
2 / 8 / 2x 18 ml	Washing Buffer WSH (conc.)	RT
6 / 30 / 2 x 66 ml	Washing Solution WST (conc.)	RT
6 / 25 / 110 ml	Elution Buffer	RT
10 / 50 / 5x 50	Mini spin columns	RT
10 / 50 / 5x 50	1.5 ml Elution tubes	RT
50 / 5x 50 / 25x 50	2 ml Collection tubes	RT

- Lyophilized **Proteinase K** should be stored at +4 °C. Prior to use, dissolve Proteinase K in 2x 0.3 / 2x 1.5 / 6x1.5 ml sterile, distilled water as given below. Dissolved Proteinase K should be stored in aliquots at 4-8 °C. Avoid repeated freeze&thaw cycles for each tube.
- **RNase A** should be stored at -20 °C

The ROTI®Prep gDNA Mini 2.0 Kit should be stored dry, at room temperature (14-25 °C). Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming. This kit is stable for at least 6 months after receipt, when stored as directed.

Contents of this Kit may not be bought separately.

The user is responsible to validate the performance of the ROTI®Prep gDNA Mini 2.0 Kit for any particular use, since the performance characteristics of our kits have not been validated for any specific application.

3.2. Required Material and Equipment not included in this kit

- Ethanol 96-99.8 %, e.g. Ethanol p.a. (e.g. 9065.1)
- Sterile, nuclease-free ddH₂O (e.g. 1HPE.1)
- 1x PBS Buffer (e.g. 9143.1)
- TE buffer (e.g.1052.1)
- Yeast Digest Buffer:
50 mM potassium phosphate (e.g.6878.1), 10 mM DTT (e.g. 6908.1), pH 7.5
- Zymolyase (10 U/μl) for enzymatic lysis of yeast cells (e.g. 9324.1)
- Lysozym (400 U/μl) for enzymatic lysis of bacteria (e.g. 8259.1)

4. Application

ROTI®Prep gDNA Mini 2.0 is designed for isolation of high-purity genomic DNA from various source material. Spin column-based preparation allows elution in a small volume of low-salt buffer, eliminating time-consuming phenol-chloroform extraction or alcohol precipitation. The columns may be used either in micro-centrifuges or on vacuum manifolds.

The kit allows the extraction of **up to 65 μg** gDNA per preparation from microbial cell cultures (max. 5x10¹⁰), fungi and eukaryotic cell cultures (max. 5x10⁶), up to 40 mg of rodent tails (0.5 – 1 cm), buccal swabs, Tissue samples (max. 20 mg) or Blood (200-400 μl).

During lysis, the sample has to be mixed carefully and, if possible, constantly. Reduced movement of the lysis mix will reduce the lysis efficiency, and, subsequently, the recovery rate of gDNA. We recommend using a shaking platform or thermomixer in order to keep the sample at constant movement.

Store the extracted DNA at +4 °C. For long time storage placing at -20 °C is recommended.

For centrifugation we recommend a standard microcentrifuge. Centrifugation steps should be carried out at room temperature.

Avoid repeated freeze&thaw cycles for the tissue to be extracted.

4.1. Before start, be sure to...

- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Buffer WSH (conc.) as follows:
1YTK.1 (10 Preps): 18 ml (20 ml final vol.)
1YTK.2 (50 Preps): 72 ml (80 ml final vol.)
1YTK.3 (250 Preps): 162 ml to each bottle (2x 200 ml final vol.)
Mix thoroughly and keep the bottle always firmly closed!

- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Solution WST (conc.) as follows:
 1YTK.1 (10 Preps): 14 ml (20 ml final vol.)
 1YTK.2 (50 Preps): 70 ml (100 ml final vol.)
 1YTK.3 (250 Preps): 154 ml to each bottle (2x 220 ml final vol.)
 Mix thoroughly and keep the bottle always firmly closed!
- **Dissolve Proteinase K** by addition of distilled H₂O as follows:
 1YTK.1 (10 Preps): 0.3 ml
 1YTK.2 (50 Preps): 1.5 ml
 1YTK.3 (250 Preps): 1.5 ml to each tube
 Mix thoroughly!
- Heat one thermal mixer or water bath to 56 or 60 °C.
- Pre-fill the needed amount of RNase free water or Elution Buffer into a 2.0 ml reaction tube and incubate at 60 °C until the elution step.
- For Lysis steps we recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

4.2. Workflow

The workflow has been provided on a separate page, in order to use a copy for in-process protocol or filing in your personal lab files.

- DNA isolation from bacterial cell cultures p. 4
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4.2.1 DNA isolation from bacterial cell cultures

Step (RT = room temperature)	done
1. Collection of bacterial cells	
Transfer the bacterial cell culture into a 2.0 ml reaction tube	
Pellet cells by centrifugation at 3.000 g (or 5000 rpm) for 10 mins and discard the supernatant	
Resuspend the bacterial cell pellet in 100 µl TE-Buffer.	
2.1 Lysis of bacterial cells	
Add 20 µl Lysozyme (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking (obligatory step for gram-positive bacteria, optional step for gram-negative bacteria). For <i>Staphylococcus</i> 10 µl Lysostaphin (0.4 U/µl) are recommended	
Add 280 µl Lysis Buffer LSV and 20 µl Proteinase K to the sample and vortex the sample shortly	
Incubate the sample for 30 minutes at 60 °C and 550 rpm in a thermoshaker.	
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 2 µl of a RNase A stock solution (10 mg/ml) to the sample vortex shortly and incubate for 10 minutes at room temperature.	
Add 200 µl Binding Buffer BSN to the sample. Mix by vortexing or pipetting until a <i>homogenous</i> solution is achieved.	
2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube	
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Add 300 µl of Washing Buffer WST to the Spin Column	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50-200 µl RNase free Water or Elution Buffer EB to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.2 DNA isolation from yeast cell cultures

Step (RT = room temperature)	done
1. Collection of yeast cells	
Pellet cells by centrifugation at 5000 g (or 7000 rpm) for 10 mins and discard the supernatant	
Resuspend the yeast cell pellet in 120 µl Yeast Digest Buffer	
2.1 Lysis of yeast cells	
Add 10 µl Zymolyase (10 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.	
Add 280 µl Lysis Buffer LSV and 20 µl Proteinase K to the sample and vortex the sample shortly	
Incubate the sample for 30 minutes at 60 °C and 550 rpm in a thermoshaker.	
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 2 µl of a RNase A stock solution (10 mg/ml) to the sample vortex shortly and incubate for 10 minutes at room temperature.	
Add 200 µl Binding Buffer BSN to the sample. Mix by vortexing or pipetting until a <i>homogenous</i> solution is achieved.	
2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube	
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Add 300 µl of Washing Buffer WST to the Spin Column	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-400 µl RNase free Water or Elution Buffer EB to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.3 DNA isolation from cell cultures

Step (RT = room temperature)	done
1. Cell lysis	
Pellet cells by centrifugation at 5.000 g (or 7.500 rpm) for 10 mins and discard the supernatant.*	
Add 100 µl 1 x PBS Buffer and resuspend the cell pellet completely by pipetting up and down several times.	
Add 300 µl Lysis Buffer LSV and 25 µl Proteinase K and mix vigorously by pulsed vortexing for 5 s. Incubate at 56 °C until the sample is completely lysed (appr. 15 – 30 minutes depends on number of cells).	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl Binding Buffer BSN to the sample. Mix by vortexing or pipetting until a <i>homogenous</i> solution is achieved.	
2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube***	
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 300 µl of Washing Buffer WST to the Spin Column	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-400 µl RNase free Water or to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.4 DNA isolation from rodent tails or tissue samples

Step (RT = room temperature)	done
1. Cell lysis	
Cut max. 40 mg of tissue sample into small pieces and place the tissue into a 1.5 ml reaction tube.	
Add 400 µl Lysis Buffer LSV and 25 µl Proteinase K and mix vigorously by pulsed vortexing for 5 s. Incubate at 56 °C until the sample is completely lysed (appr. 15 – 30 minutes depends on number of cells). <i>Important: The lysis step should be finished if the material is completely lysed. Optional centrifuge the 1.5 ml reaction tube at maximum speed for 3 minutes to spin down unlysed material. Transfer the supernatant carefully into another 1.5 ml tube.</i>	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl Binding Buffer BSN to the sample. Mix by vortexing or pipetting until a <i>homogenous</i> solution is achieved.	
2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BSN to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube	
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 300 µl of Washing Buffer WST to the Spin Column	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-400 µl RNase free Water or Elution Buffer EB to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.5 DNA isolation from buccal swaps

Note: For a maximum yield of DNA, the swab has to remain in the tube during the whole lysis procedure. We recommend to cut the shaft of the swab, in order to enable closing of the cap. Removal of the swab from the tube ahead of time will lead to a dramatically reduced yield!

Step (RT = room temperature)	done
1. Cell lysis	
Place the swab into a 1.5 ml or 2.0 ml reaction tube and cut the shaft.	
Add 400 µl Lysis Buffer LSV and 25 µl Proteinase K and mix vigorously by pulsed vortexing for 5 s. Incubate at 50 °C under continuous shaking (appr. 10- 15 minutes)	
After lysis time remove the swab from the tube and squeeze the swab on the wall of the tube to remove all Lysis Solution CBV from the swab.	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl Binding Buffer BR to the sample. Mix by vortexing or pipetting until a <i>homogenous</i> solution is achieved.	
2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of sample/Binding Buffer BR to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube***	
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of Washing Buffer WST to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 300 µl of Washing Buffer WST to the Spin Column	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-400 µl RNase free Water or Elution Buffer EB to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

4.2.6 DNA isolation from blood samples

Note: DNA isolation from blood samples is either done with exactly **200 µl** or with exactly **400 µl** of blood sample. If your sample is less than 200 or 400 µl, add 1x PBS buffer to bring it to the appropriate volume.

Step (RT = room temperature)	done
1. Cell lysis	
Pipette 200 or 400 µl of whole blood sample into a 1.5 ml reaction tube. If the sample volume is less than 200 or 400 µl, add the appropriate volume of PBS.	
Add 200 / 400 µl Lysis Buffer LSN and 20 / 30 µl Proteinase K respectively, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.	
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample, vortex shortly and incubate for 5 minutes at room temperature.	
Add 350 / 700 µl Binding Buffer BR to the sample. Mix by sorrowly pipetting up and down until a <i>homogenous</i> solution is achieved. Important: <i>Don't vortex the sample</i>	
2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply 750 µl of sample/Binding Buffer BR mix to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through. Repeat this step with the rest of you sample (only necessary in case of 400 µl blood sample).	
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 400 µl of Washing Solution WSO to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube	
Place the Spin Column into a new 2 ml collection tube	
Add 600 µl of Washing Buffer WSH to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube***	
Place the Spin Column into a new 2 ml collection tube	
Add 600 µl of Washing Buffer WSH to the Spin Column	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl RNase free Water or Elution Buffer EB to the centre of the membrane	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA. <i>Two elution steps with equal volumes of pre-warmed RNase free water or Elution Buffer (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.</i>	

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
1. Clogged spin filter	
Insufficient lysis and/or too much starting material	Increase lysis time.
	Increase centrifugation speed.
	After lysis centrifuge the lysate to pellet unlysed material.
	Check storage conditions and usage of Proteinase K. Optionally replace Proteinase K by a fresh lot.
	Reduce amount of starting material. Overload of filters reduces yield.
2. Low recovery	
Insufficient lysis	See above
Insufficient mixing with Binding Buffer BSN	Mix sample very well with Binding Buffer BSN by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter
Low amount of recovered gDNA.	Add the Elution Buffer directly onto the centre of the Spin Column.
	Prolong the incubation time with Elution Buffer.
	Increase volume of Elution Buffer used or repeat elution step.
Low concentration of eluted gDNA	Reduce the volume of Elution Buffer used. Optionally: prolong elution / incubation time.
3. Degraded or sheared DNA	
Incorrect storage of starting material.	Make sure that the starting material is frozen immediately in liquid N ₂ or in minimum at -20 °C, and is stored continuously at -80 °C!
	Avoid repeated freezing and thawing of the starting material.
Old material	Use fresh material. Check and improve storage conditions.
4. RNA contamination	
Unsufficient RNase digestion during lysis	Add RNase A during lysis.
	Check storage conditions and usage of RNase A. Optionally replace RNase A by a fresh lot.

Ordering information:
(for detailed kit content see Table under 1.)



ROTI®Prep gDNA Mini 2.0	10 preps	1YTK.1
ROTI®Prep gDNA Mini 2.0	50 preps	1YTK.2
ROTI®Prep gDNA Mini 2.0	250 preps	1YTK.3

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