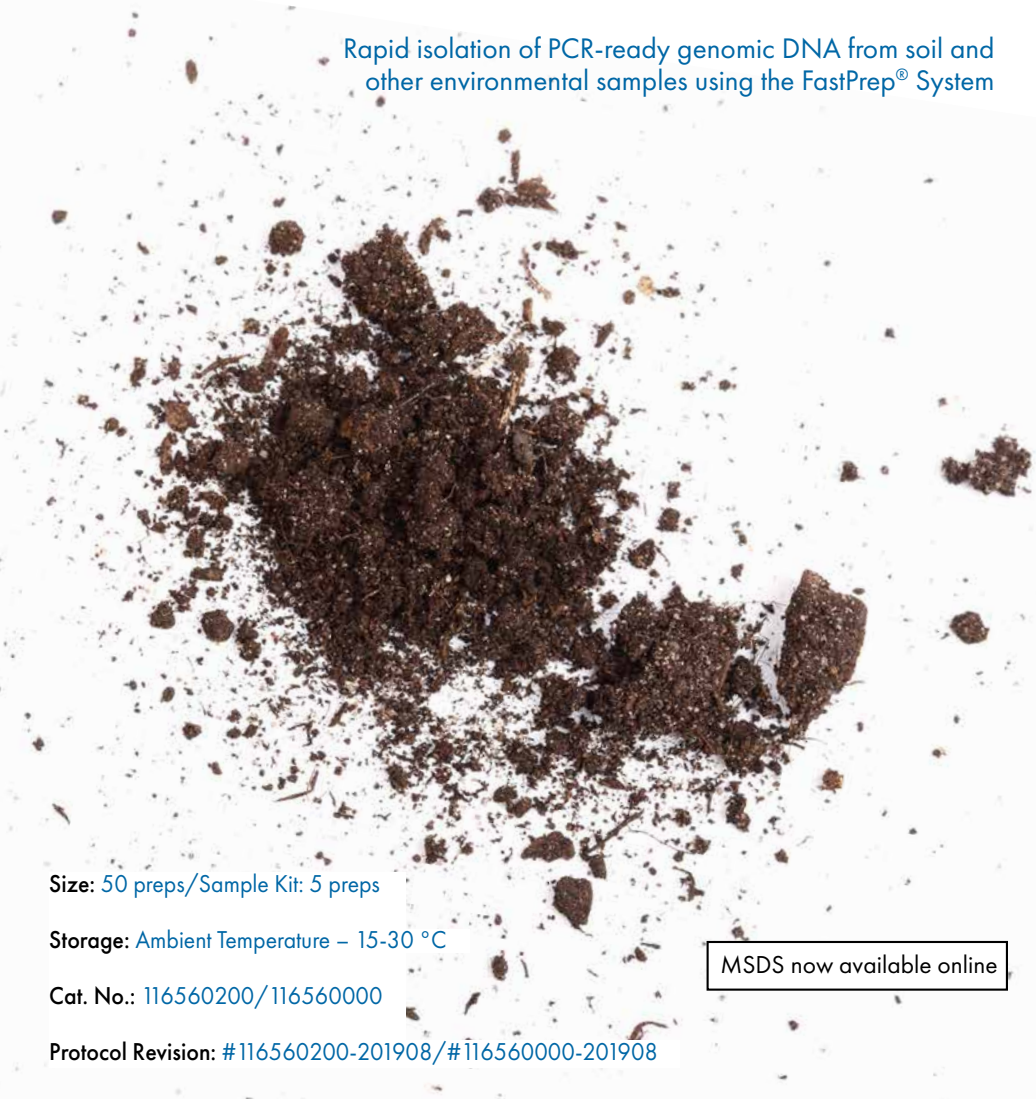
Instruction Manual

FastDNA™ SPIN Kit for Soil

Rapid isolation of PCR-ready genomic DNA from soil and other environmental samples using the FastPrep® System



Size: 50 preps/Sample Kit: 5 preps

Storage: Ambient Temperature – 15-30 °C

Cat. No.: 116560200/116560000

Protocol Revision: #116560200-201908/#116560000-201908

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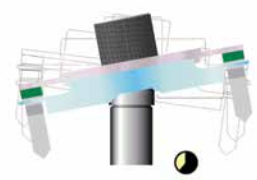
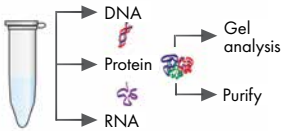
HOMOGENIZATION



EXTRACTION



Instruments

FastPrep-24™ 5G

FastPrep-96™

Super FastPrep-2™

Adapters

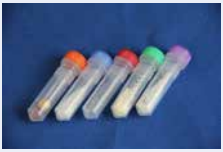
Metal

Cryogenic

High Throughput

Large Sample Volume

Lysing Matrix Tubes



2 mL

4.5 mL

15 mL

50 mL

Extraction Kits

DNA

RNA

Protein

Rapid Isolation of PCR-Ready Genomic DNA

From Soil and Other Environmental Samples

- **Size:** 50 preps/Sample Kit: 5 preps
- **Storage:** Ambient Temperature – 15-30 °C
- **Cat. No.:** 116560200/116560000
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Table of Contents

1. Introduction to FastDNA™ SPIN Kit for Soil and the FastPrep Instruments

The FastDNA™ SPIN Kit for Soil quickly and efficiently isolates PCR-ready genomic DNA directly from soil samples in less than 30 minutes. Designed for use with the FastPrep® instruments from MP Biomedicals, plant and animal tissues, bacteria, algae, fungal spores and other members of a soil population are easily lysed within 40 seconds. These benchtop devices use a unique, optimized motion to homogenize samples by multidirectional, simultaneous impaction with lysing matrix particles. FastPrep® instruments provide an extremely quick, efficient and highly reproducible homogenization that surpasses traditional extraction methods using enzymatic digestion, sonication, blending, douncing and vortexing.

Samples are placed into 2.0 mL tubes containing Lysing Matrix E, a mixture of ceramic and silica particles designed to efficiently lyse all soil organisms, including historically difficult sources, such as eubacterial spores and endospores, gram positive bacteria, yeast, algae, nematodes and fungi. Homogenization in a FastPrep® instrument with Lysing Matrix E takes place in the presence of MT Buffer and Sodium Phosphate Buffer, reagents carefully developed to protect and solubilize nucleic acids and proteins upon cell lysis. These reagents work synergistically to allow extraction of genomic DNA with minimal RNA contamination.

Following lysis, samples are centrifuged to pellet soil, cell debris and lysing matrix. DNA is purified from the supernatant with a silica-based GeneClean® procedure using SPIN filters. Eluted DNA is ready for PCR, restriction digest, electrophoresis and any other desired application.

2. Kit Components and User Supplied Materials

2.1 FastDNA SPIN Kit for Soil Components

Product	Kit Size	Cat. No.	Sample Kit Size	Sample Cat. No.
Lysing Matrix E	50 x 2.0 mL tubes	116914050	5 x 2 mL tubes	116914005
Sodium Phosphate Buffer	60 mL	116560205	6 mL	116560005
MT Buffer	8 mL	116511202	800 µL	116511002
PPS Solution	25 mL	116560203	2.5 mL	116540003
Binding Matrix	2 x 30 mL	116540408	6.6 mL	116540008
SPIN Modules	50 each	116560210	5 each	116560010
Catch Tubes	50 each	116560211	5 each	116560011
Concentrated SEWS-M	12 mL	116540405	1.2 mL	116540005
DES	20 mL	116540406	2 mL	116540006
BBS Gel Loading Dye	200 µL	16540407	20 µL	116540007
User manual	1 each	-	1 each	-
Certificate of Analysis	1 each	-	1 each	-

2.2 User Supplied Materials

- FastPrep instrument *(see Section 9)*
- Microcentrifuge that can freely spin 2.0 mL tubes
- Microcentrifuge tubes (2.0 mL and 1.5 mL)
- Clean 15 mL tubes for DNA binding
- Rotator or low-speed vortex

3. Important Considerations Before Use

3.1 Preparation of SEWS-M Wash Solution

The FastDNA SPIN Kit for Soil contains a bottle with 12 mL *(sample kit: 1.2 mL)* of a concentrated SEWS-M wash solution. Before using this solution, add 100 mL *(sample kit: 10 mL)* of 100% ethanol and mark on the bottle label the date ethanol was added. Ensure that the bottle is securely closed to prevent evaporation, and store at room temperature.

3.2 Sample Lysis with the FastPrep® Instrument

The fill volume of the lysing matrix tube after addition of Sodium Phosphate and MT Buffers to the sample should allow sufficient air space in the sample tube for efficient FastPrep instrument processing. MP Biomedicals recommends using up to 500 mg of most soil types. Very wet soils or detritus-rich soils may require less sample by mass. Ensure that there is 250 – 500 µL of empty space in the tube. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

MP Bio's Lysing Matrix particles and tubes have been rigorously tested and validated in the FastPrep instrument. The use of other products with the FastPrep instrument is not recommended and may result in sample loss or instrument failure. A single 40 second run at a speed setting of 6.0 in the FastPrep instrument is sufficient to lyse almost all samples. If the user experimentally determines that additional processing time is required, the sample should be incubated on ice in the Lysing Matrix E tube for at least 2 minutes between successive FastPrep instrument homogenizations to prevent overheating the sample and tube.

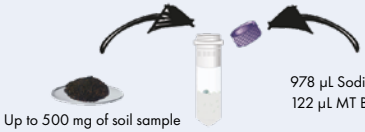
4. Safety Precautions

Binding Matrix contains components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucous membranes (gloves, lab coat, and eye protection). Consult the **Material Safety Data Sheet** found online at www.mpbio.com.

5. Protocol

5.1 FastDNA™ SPIN Kit for Soil Typical Work Flow

1. Prepare the Sample



2. Homogenize with the FastPrep instrument *(or similar instrument)*

40 sec

Load tube in FastPrep instrument.
Process: 40 s at a speed setting of 6.0 m/s.

5 - 10 mins
14,000 g

Centrifuge to pellet debris.

3. Precipitate proteins

5 mins
14,000 g

Transfer supernatant to a clean 2 mL microcentrifuge tube. Add 250 µL PPS and mix 10 times.

Centrifuge to pellet precipitate.

4. Adjust binding conditions

15 mL tube

Transfer supernatant to 15 mL tube.
Add 1 mL Binding Matrix Solution.
Invert 2 mins and place tube on a rack for 3 mins. Discard 500 µL of supernatant.

5. Bind the DNA

1 min
14,000 g

Transfer max 600 µL of DNA Solution to a SPIN™ Filter Tube. Empty catch tube.
Repeat step 5 if the volume of the mixture is higher than 600 µL.

6. Wash the SPIN™ Filter

1 min
14,000 g

Add 500 µL prepared SEWS-M Solution.
Empty catch tube.

7. Dry the SPIN™ Filter

2 min
14,000 g

Air dry SPIN™ Filter for 5 mins at room temperature.

8. Elute the DNA

Use a new catch tube

1 min
14,000 g

50-100 µL DES Elution Solution.
DNA in the catch tube is ready-to-use.

5.2 FastDNA™ SPIN Kit for Soil Detailed Protocol

1. Add up to 500 mg of soil sample to a Lysing Matrix E tube.

   NOTE: See section 3.2 for other important guidelines

2. Add 978 µL Sodium Phosphate Buffer to sample in Lysing Matrix E tube.
3. Add 122 µL MT Buffer.

   What's Happening: Begin to solubilize membrane proteins with detergents as well as extra-cellular proteins and contaminants in soil.

4. Homogenize in the FastPrep instrument for 40 seconds at a speed setting of 6.0.

   What's Happening: Mechanical disruption of cell walls of soil organisms and releasing nucleic acids into the protective buffer.

5. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.

   NOTE: Extending centrifugation to 15 minutes can enhance elimination of excessive debris from large samples or from cells with complex cell walls.

   What's Happening: Pellet insoluble cellular material and lysing matrix particles.

6. Transfer supernatant to a clean 2.0 mL microcentrifuge tube. Add 250 µL PPS (Protein Precipitation Solution) and mix by inverting the tube 10 times.

   What's Happening: Separate the solubilized nucleic acids from the cellular debris and lysing matrix. Flocculation of protein-containing micelles.

7. Centrifuge at 14,000 x g for 5 minutes to pellet precipitate. Transfer supernatant to a clean 15 mL microcentrifuge tube.

   NOTE: While a 2.0 mL microcentrifuge tube may be used at this step, more efficient mixing and DNA binding will occur in a larger tube.

   What's Happening: Removal of flocculated proteins.

8. Resuspend the Binding Matrix suspension and add 1.0 mL to the supernatant in the 15 mL tube.

9. Place on rotator or invert by hand for 2 minutes to allow binding of DNA. Place tube on a rack for 3 minutes to allow settling of silica matrix.

   What's Happening: Nucleic acids bind to the silica matrix in the presence of chaotropic salts.

10. Remove and discard 500 µL of supernatant being careful to avoid settled Binding Matrix.

11. Gently resuspend Binding Matrix in the remaining amount of supernatant. Transfer approximately 600 µL of the mixture to a SPIN™ filter and centrifuge at 14,000 x g for 1 minute. Empty the catch tube and add the remaining mixture to the SPIN Filter and centrifuge as before. Empty the catch tube again.

12. Add 500 µL prepared SEWS-M and gently resuspend the pellet using the force of the liquid from the pipet tip.

    NOTE: *Ensure that ethanol has been added to the Concentrated SEWS-M. See section 3.1.*

    What's Happening: Continuing to solubilize proteins.

13. Centrifuge at 14,000 x g for 1 minute. Empty the catch tube and replace.

    What's Happening: Desalting with ethanol and additional detergents remove impurities by centrifuging through the SPIN Filter bucket while the purified DNA is still bound to the silica.

14. Without addition of any liquid, centrifuge a second time at 14,000 x g for 2 minutes to "dry" the matrix of residual wash solution. Discard the catch tube with a new, clean catch tube.

15. Air dry the SPIN Filter for 5 minutes at room temperature.

    What's Happening: Removal of residual ethanol.

16. Gently resuspend Binding Matrix (above the SPIN Filter) in 50 - 100 µL of DES (DNase/Pyrogen-Free Water).

    NOTE: *To avoid over-dilution of the purified DNA, use smallest amount of DES required to resuspend Binding Matrix pellet*

    NOTE: *Yields may be increased by incubation for 5 minutes at 55°C in a heat block or water bath.*

    What's Happening: Purified nucleic acids elute from the silica with collapse of cation bridges because low salt elution solution rehydrates both the silica and the DNA.

17. Centrifuge at 14,000 x g for 1 minute to bring eluted DNA into the clean catch tube. Discard the SPIN Filter. DNA is now ready for PCR and other downstream applications. Store at -20°C for extended periods or 4°C until use.

DNA Analysis by Agarose Gel Electrophoresis

Load 5 µL of the eluted DNA per well on a 1% agarose gel in 0.5 x TBE electrophoresis buffer.

BBS loading dye, a 10X solution of bromophenol blue and sucrose solution, is supplied for this application.

6. Table of Recommended FastPrep® Settings

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep Speed	FastPrep Time
Soil and Sediment					
Sediment	Soil/Rock	50 mg	E	5.5	2 x 30 sec
Soil	Sandy Sample	50 mg	E	4.0	4 x 30 sec
Soil	Litter	50 mg	E	5.5	30 sec
Soil	Brunisol Dark Gray Luvisol	50 mg	E	5.5	40 sec
Soil	Soil from Grassland	50 mg	E	5.5	2 x 30 sec
Soil	Rhizosphere	50 mg	E	6.0	40 sec
Sediment	Marine Sediment	50 mg	E	5.5	2 x 40 sec
Soil	Asphalt-permented Soil	50 mg	E	6.0	40 sec
Bacteria					
Listeria monocytogenes	Cells	10^9 cells	B	6.0	3 x 30 sec
Streptococcus pyogenes	Cells	10^9 cells	B	6.0	20 sec
Streptococcus mutants	Cells	10^9 cells	B	6.0	30 sec
Streptococcus aureus	Cells	10^9 cells	B	6.0	2 x 40 sec
Photorhabdus luminescens	Cells	10^9 cells	B	6.0	2 x 40 sec
Escherichia coli	Cells	10^9 cells	B	6.0	30 sec
Mycobacterium tuberculosis	Cells	10^8 cells	B	6.0	2 x 45 sec
Lactococcus lactis	Cells	10^8 cells	B	6.0	3 x 30 sec
Yeast & Fungi					
Saccharomyces cerevisiae	Cells	2 x 10^8 cells	C	6.0	40 sec
Schizosaccharomyces pombe	Cells	10^8 cells	C	5.0	4 x 15 sec
Candida albicans	Cells	10^8 cells	C	6.0	2 x 30 sec
Cryptococcus neoformans	Cells	10^8 cells	C	6.0	4 x 30 sec
Aspergillus fumigatus	Cells	10^8 cells	C	6.0	2 x 30 sec
Fusarium solani	Cells	10^8 cells	C	6.0	2 x 30 sec

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep Speed	FastPrep Time
Plants					
Alpowa Wheat	Leaf Tissue	75 mg	D	6.0	40 sec
Alpowa Wheat	Seed	100 mg	A	6.0	40 sec
Arabidopsis thaliana	Fresh Leaves	50 mg	D	6.0	40 sec
Arabidopsis thaliana	Fresh Leaves	200 mg	D	6.0	2 x 40 sec
Bartlett Pear	Leaf Tissue	50 mg	D	6.0	40 sec
Classic Oat	Leaf Tissue	75 mg	D	6.0	40 sec
Classic Oat	Seed	100 mg	A	6.0	40 sec
Corn	Leaf Tissue	100 mg	D	6.0	40 sec
Crest Barley	Leaf Tissue	100 mg	D	6.0	40 sec
Crest Barley	Root	300 mg	A	6.0	40 sec
Kaybonnet Rice	Leaf Tissue	100 mg	D	6.0	40 sec
Kaybonnet Rice	Seed	100 mg	A	6.0	40 sec
Klages Barley	Root	300 mg	A	6.0	40 sec
Klages Barley	Leaf Tissue	70 mg	D	6.0	40 sec
Tobacco	Leaf Tissue	75 mg	D	6.0	40 sec
Laffite Rice	Leaf Tissue	75 mg	D	6.0	40 sec
Laffite Rice	Sprout Leaf	100 mg	D	6.0	2 x 30 sec
Soybean	Seed	100 mg	A	6.0	40 sec
Corn	Seed	100 mg	A	6.0	40 sec
Oat FL 502	Leaf Tissue	75 mg	D	6.0	40 sec
Oat FL 502	Seed	100 mg	A	6.0	40 sec
Riser Oat	Leaf Tissue	70 mg	D	6.0	40 sec
Richland Soybean	Leaf Tissue	100 mg	D	6.0	40 sec
Tam Wheat	Leaf Tissue	75 mg	D	6.0	40 sec
Tam Wheat	Root	80 mg	A	6.0	40 sec
Tomato, Early Girl	Leaf Tissue	75 mg	D	6.0	4 x 30 sec
Williams 82 Soybean	Leaf Tissue	70 mg	D	6.0	40 sec
Wrens Rye	Leaf Tissue	100 mg	D	6.0	40 sec
Pine	Needle	100 mg	A	6.0	40 sec

7. Troubleshooting Guide

7.1 Wet Soil Sample

If the soil is extremely wet, transfer the Lysing Matrix E components to another sterile holding tube. Place the soil sample in the empty matrix tube, and centrifuge 30 seconds at 10,000 x g. Decant as much liquid as possible, replace lysing matrix components and continue with protocol.

7.2 Low DNA Yield in Eluate

1. **Insufficient Lysis:**
While a FastPrep speed setting of 6.0 m/s and a 40 second run time will be adequate for most soil types, additional processing may be necessary. If repeat processing cycles are necessary it is recommended to incubate Lysing Matrix tubes in ice for 2 minutes between cycles to avoid excessive heat build up.

2. **Insufficient Binding Matrix:**
Binding matrix is supplied as a suspension and must be completely dispersed before pipetting. Vigorously shake the bottle of binding matrix to produce a uniform suspension. In some instances, vortexing may be necessary.

3. **Ethanol Not Added to SEWS-M Solution:**
SEWS-M solution is supplied as a concentrate. 100 mL of 100% ethanol must be added to the concentrate before use.

4. **DNA Not Eluted Efficiently:**
To increase elution efficiency, after resuspending binding matrix with DES solution, incubate for 5 minutes at 55°C before centrifuging the final eluate.

7.3 DNA Does Not Amplify

1. **Quantitate DNA Yield:**
By gel electrophoresis, or spectrophotometer. Excess DNA will inhibit PCR reactions.

2. **Dilute Template DNA:**
This should not be necessary with DNA isolated with the FastDNA SPIN Kit for Soil, but is still an option.

3 **Non-Specific Bands:**
Check possibility that target DNA is in low abundance in the eluate. It is possible that some species of interest, particularly parasitic cysts and oocytes, may need additional processing or even more aggressive lysing matrix (such as Lysing Matrix A) in order to disrupt the thick protein cell wall.

4 **Verify PCR Optimization Conditions:**
Changing reaction conditions or primer selection may be necessary.

7.4 DNA Fragmented

1 **Use Care With Liquid Transfer:**
Once the DNA is bound to the binding matrix, care should be taken to avoid DNA shearing. All liquid manipulations, especially resuspension of matrix in SEWS-M solution and DES solution, should be performed gently and deliberately. The use of wide bore pipet tips is recommended for these steps.

2 **Optimize Lysis Conditions:**
High powered bead beating cell disrupters can shear DNA if process settings are too long or powerful. While FastPrep speed setting of 6.0 m/s and a 40 second runtime will be adequate for most soil types, it is possible that lowering speed and/or duration settings will result in higher MW DNA.

7.5 Low A_{260}/A_{280} Ratios for Purified DNA

1 **Ethanol Not Added to SEWS-M Solution:**
SEWS-M solution is supplied as a concentrate. 100 mL of 100% ethanol must be added to the concentrate before use.

2 **Proteins Not Removed Efficiently:**
PPS solution must be efficiently mixed in the lysate (step 6). Invert tube by hand at least 10 times, or mix by pipet pumping. A 5 minute incubation on ice can further precipitate proteins from difficult samples.

3 **Contaminants Not Removed Efficiently:**
During the wash with SEWS-M solution (step 12), it is necessary to resuspend the entire binding matrix pellet to efficiently remove contaminants. This step should be performed gently and deliberately. The use of wide-bore pipet tips is recommended for this step.

7.6 Elevated A_{230} Absorbance

1. **Proteins Not Removed Efficiently:**
PPS solution must be efficiently mixed in the lysate (step 6). Invert tube by hand at least 10 times, or mix by pipet pumping. A 5 minute incubation on ice can further precipitate proteins from difficult samples. For samples suspected to contain very high levels of inhibitors, an additional PPS treatment can be performed.

2. **Contaminants Not Removed Efficiently:**
During the wash with SEWS-M solution (step 12), it is necessary to resuspend the entire binding matrix pellet to efficiently remove contaminants. This step should be performed gently and deliberately. The use of wide-bore pipet tips is recommended for this step. For samples suspected to contain very high levels of inhibitors, an additional PPS treatment can be performed.

3. **Residual Ethanol in the Final Eluate:**
Be sure to perform an additional 2 minute centrifugation step (step 14) after all of the SEWS-M solution has ben run through the column. Performing the minute air dry (step 15) with a 60 °C incubation can also aid in removing residual ethanol.

8. References

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9. Related Products

Product Name	Cat. No.
Instruments	
FastPrep-24™ Classic	116004500
FastPrep-24™ 5G	116005500
FastPrep-96™	116010500
SuperFastPrep-2™	116012500
MPure-12™	117002200
Kits	
FastDNA™ Kit	116540400
FastRNA™ Pro Blue Kit (Bacteria)	116025050
FastDNA™ SPIN Kit	116540600
FastDNA™ SPIN Kit for Feces	116570200
FastRNA™ Pro Soil-Direct Kit	116070050
FastRNA™ Pro Soil-Indirect Kit	116075050
FastRNA™ Pro Green Kit (Plant & Animal)	116045050
FastRNA™ Pro Red Kit (Yeast & Fungus)	116035050
FastPROTEIN™ Blue Matrix	116550400
FastPROTEIN™ Red Matrix	116550600

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Notes:

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