BIO LINKER

QUICK PROTOCOL
BIOSCRIPT REVERSE TRANSCRIPTASE

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BioLinker's Bioscrip Reverse Transcriptase™ is an engineered version of MMLV RTx with more thermal stability and increased specificity by reduced RNAse H activity mutated domain [1]. The enzyme can be used to synthesize first-strand cDNA at temperatures up to 55°C, providing higher yields of cDNA, and more fulllength product than other reverse transcriptases. It can generate cDNA from 100 bp to >12 kb.

BIOSCRIPT REVERSE TRANSCRIPTASE COMPONENTS

- BioScript RTx™: 10,000 U/mL (200 U/µl)
- Buffer RT 5X* 1000 µl

*250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM MgCl2
**RNA inhibitor not included

UNIT DEFINITION

One unit incorporates 1 nmol of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo(dT)25 as template-primer (3).

STORAGE BUFFER

Storage Buffer: 40 mM Tris-HCl (pH 7.5), 200 mM NaCl, 100 mM DTT, 0.01% (v/v) tween 20, 50% (v/v) glycerol

STORAGE CONDITIONS

Store all components at -20°C (non-frost-free). Thaw Buffer RT 5x at room temperature just prior to use and refreeze immediately.

References 1. Kotewicz, M.L., D'Alessio, J.M., Driftmier, K.M., Blodgett, K.P., and Gerard, G.F. (1985) Gene 35, 249.

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STEP 01: Compound and Primers Mix

Add the following components to a nuclease-free microcentrifuge tube:

- 1 µl of oligo(dT)20 (50 µM); or 200–500 ng of oligo(dT)12-18; or 50–250 ng of random primers; or 2 pmol of gene-specific primer
- 10 pg–5 µg total RNA or 10 pg–500 ng mRNA
- 1 µl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)
- Sterile, distilled water to 13 µl

STEP 02: Mixture an Incubation

Heat mixture to 65°C for 5 minutes
Incubate on ice for at least 1 minute

STEP 03: Collect Content and Additions

Collect the contents of the tube by brief centrifugation and add:

4 µl Buffer 5X RT 1 µl Recombinant RNase Inhibitor.

Note: When using less than 50 ng of starting RNA, the addition of RNAse inhibitor is essential.

1 µl of BIOScript™ RTx (200 units/µl)*

*If generating cDNA longer than 5 kb at temperatures above 50°C using a gene-specific primer or oligo(dT)20, the amount of BioScript RT may be raised to to 400 U (2 µl) to increase yield.

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STEP 04: Mix and spin

Mix by pipetting gently up and down and spin the tube
If using random primers, incubate tube at 25°C for 5 minutes.

STEP 05: Incubation

Incubate **at 50°C for 30–60 minutes.**

Increase the reaction temperature **to 55°C for gene-specific primer.**

Reaction temperature **may also be increased to 55°C for difficult templates** or templates with high secondary structure.

STEP 06: Inactivation

Inactivate the reaction by heating at 70°C for 15

The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (those >1 kb) may require the removal of RNA complementary to the cDNA.

To remove RNA complementary to the cDNA, add 1 µl (2 units) of E. coli RNase H and incubate at 37°C for 20 minutes.

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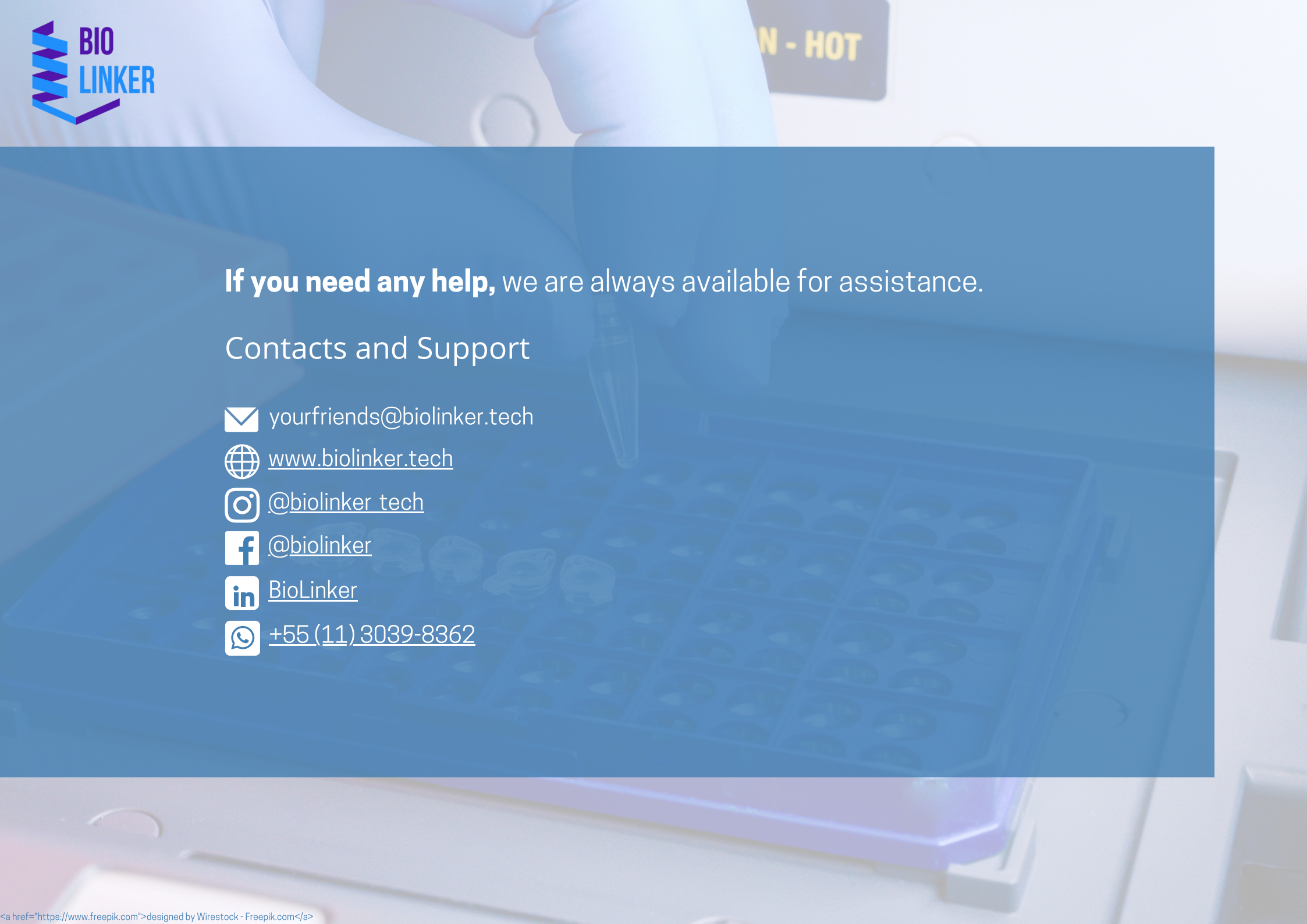
REACTION PROTOCOL

Recommended Setup Reaction:

Components	Volume
5X Buffer RT [	4µL
10 mM dNTP Mix	1µL
Sense primer (10 µM)*	0.5µL
Antisense primer (10 µM)*	0.5 µL
BIOSCRIPT reverse transcriptase (200U/µl)	1 µL
RNAse Inhibitor	1µL
RNA template (20pg-1000 ng)	1-12µL
Autoclaved, distilled water **(complete till 20ul)	-µL
TOTAL VOLUME	**To 20 µL**

*Primers concentration should be optimized
** DEPC water and ultrapure autoclaved water are recomendaded



If you need any help, we are always available for assistance.

Contacts and Support

yourfriends@biolinker.tech

www.biolinker.tech

@biolinker_tech

@biolinker

BioLinker

+55 (11) 3039-8362