AnyGenes®

Efficient technologies for signaling pathways

# SpeAmp$^{n}$ cDNA Pre-amplification Kits

# User Guide

- ✓ Cat # SpeAmp$^{n}$-10
- ✓ Cat # SpeAmp$^{n}$-25
- ✓ Cat # SpeAmp$^{n}$-50

To use with our range of pathway-specific primer pools :

- ✓ Cat # Prim$^{nx}$-10 **XXXX**
- ✓ Cat # Prim$^{nx}$-25 **XXXX**
- ✓ Cat # Prim$^{nx}$-50 **XXXX**

*** XXXX : name of the selected specific pathway**

Compatible with all AnyGenes® SignArrays products

**For research use only**

**Store at -20°C & keep away from light**

**2018**

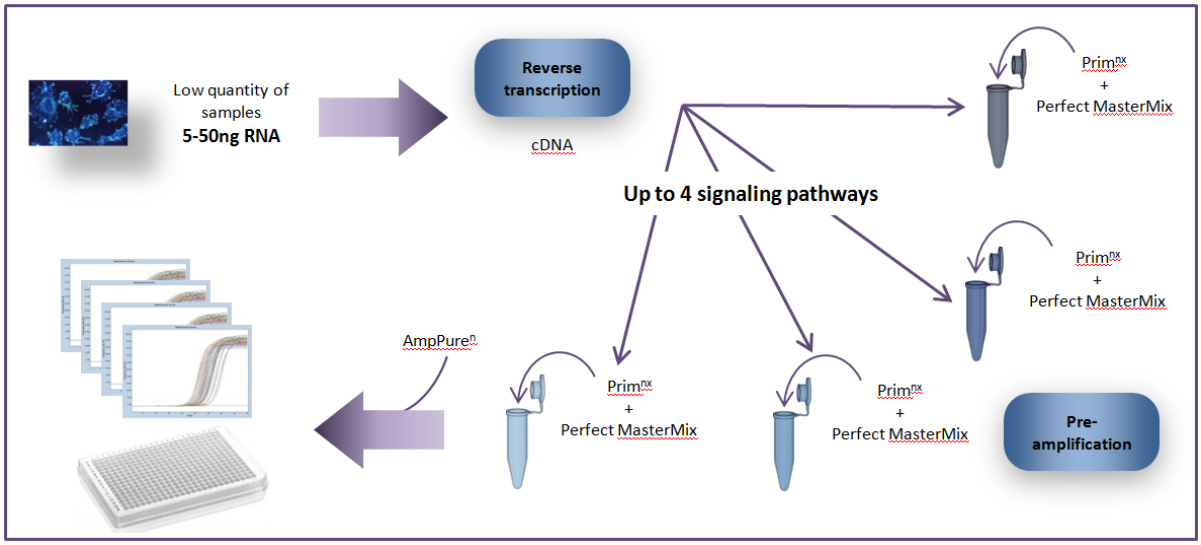
## Summary

# I. Product information

## 1) Introduction

The **SpeAmp$^n$ system** is an optimised and complete solution allowing cDNA pre-amplification to perform high-throughtput transcriptomic analysis with only small amounts of biological material (5-50ng RNA). SpeAmp$^n$ kits are fully compatible with the AnyGenes® SignArrays® system and you can now analyse **4 signaling pathways of your choice with only 5 to 50ng RNA**. This step can be necessary with precious samples to get sufficient material to perform such analysis and avoid gene expression values to close to the detection limit.

**Principle :**




The SpeAmp$^n$ system has been **optimised and experimentally validated** thanks to our strict quality control policy in order to give **highly reliable and reproducible results**.

It contains :

- **Perfect Master Mix (PM)**, containing a thermo-stable Taq DNA Polymerase as well as buffer and $MgCl_2$ at concentrations optimised for the high performance of the enzyme and dNTPs required for amplification of DNA targets by PCR

- **AmpPure$^n$**, containing a pool of enzymes allowing purification of the PCR products at the end of the pre-amplification step.

The cDNA pre-amplification step requires the use of the following kit :

- **Prim$^{nx}$ (not included in the SpeAmp$^{n}$ kit)**, which is a pool of specific primers compatible with the SignArrays of your choice.

The cDNA pre-amplification step requires only the addition of your template (cDNA) and Prim$^{nx}$ primer pool to the Perfect Master Mix. The performance of AnyGenes® Perfect Master Mix has been carefully designed to provide you a high sensitivity and specificity, without any bias in comparison with the non-pre-amplification protocol. For any details, see www.anygenes.com.

✓ **Quality Control**

As part of our routine quality assurance program, all AnyGenes® products are monitored to ensure the highest levels of performance and reliability.

## 2) Intended use & licencing

For molecular biology research use only. This kit is not intended for diagnosis, prevention or therapeutic applications. AnyGenes® will be not responsible of the misuse of their products.

Purchase of AnyGenes® kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others.

## 3) Kit contents

The SpeAmp$^{n}$ system is comprised of :

- Perfect Master Mix, supplied in 2X concentration. This Master Mix is optimised to use for cDNA pre-amplification conditions and it contains:
  - Optimised buffer components including $MgCl_2$
  - Hot Start Taq DNA Polymerase
  - dNTPs

- AmpPure$^{n}$ reagent, containing a pool of enzymes allowing purification of the PCR products at the end of the pre-amplification step.

These products are available in several formats compatible with all of our SignArrays® systems:

| Catalog Ref : | Contents |
|---|---|
| **SpeAmp$^{n}$-10** | **Perfect Master Mix (PM)** (1 x 0.5 mL) + **AmpPure$^{n}$** (30 µl) + **PCR grade $H_2O$** (1 x 1.5 mL) |
| **SpeAmp$^{n}$-25** | **Perfect Master Mix (PM)** (1 x 1.25 mL) + **AmpPure$^{n}$** (75 µ)l + **PCR grade $H_2O$** (2 x 1.5 mL) |
| **SpeAmp$^{n}$-50** | **Perfect Master Mix (PM)** (2 x 1.25 mL) + **AmpPure$^{n}$** (150 µl) + PCR grade $H_2O$ (4 x 1.5 mL) |

The SpeAmp$^{n}$ system needs to be use with primer pools (Prim$^{nx}$, 0.5µM for each primer pair), specific of the signaling or pathological pathways of your choice and fully compatible with the SignArrays® system:

| Catalog Ref : | Contents |
|---|---|
| **Prim$^{nx}$-10 XXXX** | **Prim$^{nx}$-10** (75 µl) |
| **Prim$^{nx}$-25 XXXX** | **Prim$^{nx}$-25** (187.5 µl) |
| **Prim$^{nx}$-50 XXXX** | **Prim$^{nx}$-50** (375 µl) |

*** XXXX : name of the selected specific pathway**

For more product information, please visit **www.anygenes.com** or contact us at **technical@anygenes.com**

## 4) Storage & stability

Upon receipt, store SpeAmp$^{n}$ and Prim$^{nx}$ kits at -20°C until their use. These storage conditions guarantee a long-term storage of AnyGenes® products for a minimum period of six months after their receipt. Moreover, in order to guarantee the stability of these products, avoid repeated freezing and thawing cycles. If small volumes of reagents are frequently required, we recommend to stock alicots at -20°C.

## 5) Additional reagents and equipment required

### A) Reagents :

- Diluted template (cDNA) from a Reverse Transcription made with 50 ng RNA
- Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* $H_2O$ (*supplied with SpeAmp$^{n}$ kits*)

**Caution :** Do not use DEPC $H_2O$ !!!

### B) Material :

- Thermal Cycler Instrument (with sufficient ramp rate (> 1°C/s))
- PCR plates centrifuge, vortex mixer and Mini-centrifuge
- “nuclease, RNase, DNase free” tips PCR plates, sealing foils and tubes
- Pipettes for reaction mix preparation and dispensing

For more product information, please visit **www.anygenes.com** or contact us at **technical@anygenes.com**

## II. Protocol

### 1) Before you start...

To obtain reliable and reproducible results and avoid contamination and false-positive signals, it is important and necessary to follow Good Laboratory Practices.

Moreover, you don't need to add $MgCl_2$ in your PCR reaction mix. The concentration of MgCl2 in our kits has already been adjusted to improve efficiency, specificity and repeatability.

**Caution: Ensure that the used cDNA correspond to a Reverse Transcription performed with only 50ng RNA. AnyGenes cannot guarantee any results if the protocol are not scrupulously followed. For starting RNA amounts higher than 50ng, the conditions of cDNA Pre-amplification will not be respected, leading to a loss of enzyme efficiency and results reliability.**

**Ensure that you are ready to perform the qPCR SignArrays directly after this cDNA Pre-amplification step before starting this protocol in order to optimize your experiments.**

### 2) Procedure

1) Thaw AnyGenes® **Perfect Master Mix, Prim$^{nx}$ reagents** and your **cDNA samples** 20 minutes before use, in order that slowly reaches room temperature. You can also work with your samples on ice.

2) Prepare the work area (highly recommended under workstation) by carefully cleaning all material and areas with a suitable detergent and then decontaminating the workstation through exposure to UV.

3) Meanwhile, briefly centrifuge tubes and reagents and prepare the cDNA Pre-amplification reaction mix in a 2 ml tube according to the following table:

| Reagents | For 25 µl final reaction volume | |
|---|---|---|
| | Volumes / reaction | For n reactions |
| 2X Perfect Master Mix | **12.5 µl** | **n x 12.5 µl** |
| Prim$^{nx}$ (primer pool 0.5µM) | **7.5 µl** | **n x 7.5 µl** |
| **Total Pre-Amp Reaction Mix Volume** | **20 µl per reaction** | **n x 20 µl per reaction to dispense** |
| cDNA template (1/4 RT) | **+ 5 µl** | **+ 5 µl** |
| **Total Reaction Volume** | **25 µl per well or tube** | **25 µl per well or tube** |

For more convenience and for high specificity and efficiency of your experiments, we have optimized our specific primers and composition of our kits so as to use together for best specific and reliable cDNA Pre-amplification PCR and qPCR results.

4) Mix thoroughly with a pipette or briefly centrifuge the **Pre-Amp Reaction Mix**.

5) According to your qPCR plate format, dispense 20 µl per well of this mix on a PCR plate or tubes, according to the compatibility of your Thermal Cycler.

**NB:** Change tips to avoid cross contamination once it is necessary.

6) Add 5 µl of cDNA **(1/4 of the non-diluted Reverse Transcription)** per well or tube.

7) Cover the plate with a suitable sealing foil or close the caps of your tubes.

**Caution:** Do not prepare your PCR mix too early to ensure reliable and reproducible results. However, if your plate was prepared before the start of the qPCR run, keep the qPCR plate on ice or at 4°C in a refrigerator.

8) Mix gently and centrifuge the plate 15-60 s at 1 000 g to remove any bubbles.

9) Meanwhile, prepare and check the run program under the following cDNA Pre-amplification PCR conditions (compatible with most PCR instruments):

| Phase | Number of cycles | Time | Temperature | Acquisition mode | Commentaries |
|---|---|---|---|---|---|
| **Initial denaturation - HOT start Taq activation** | **1** | **10 min** | **95°C** | **/** | **« Hot-start DNA Taq polymerase » activation** |
| **cDNA Pre-amplification** | **10** | **10 s** | **95°C** | **/** | **Denaturation of cDNA brands** |
| | | **30 s** | **60°C** | **quantification** | **Hybridation & elongation steps** |

***For any further information, please contact AnyGenes® technical support via technical@anygenes.com***

10) Place the plate or tubes in your Thermal Cycler.

11) Start the cDNA Pre-amplification PCR run, following the manufacturer's recommendations and protocols.

12) Once the run is complete, release the plate or tubes of your Thermal Cycler and thaw the **Ampure reagent**.

13) Release the sealing foil or open your tubes and add 3µl of Ampure to each well or tube according to the following table :

| Reagents | Volumes / reaction |
|---|---|
| Total cDNA Pre-Amp Reaction Volume | **25 µl** |
| Ampure | **3 µl** |
| **Total Purification Mix Volume** | **28 µl per reaction** |

14) Place your plate or tube in your Thermal Cycler and proceed to the Purification step according to the following table :

| Phase | Number of cycles | Time | Temperature |
|---|---|---|---|
| **Purification** | **1** | **30 min** | **37°C** |
| **Enzyme inactivation** | **1** | **20 min** | **80°C** |

**NB: The resulting Pre-amplified and Purified cDNA can be preserved at 4°C prior to perform qPCR SignArrays or overnight at -20°C.**

**To continue with the SignArrays system :**

1) Dilute your pre-amplified cDNA with Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* $H_2O$ according your qPCR SignArrays plate format :

| Reagents | Volumes / sample for the SignArrays 96 system *(20µl final qPCR reaction)* | Volumes / sample for the SignArrays 384 system *(10µl final qPCR reaction)* |
|---|---|---|
| **PreAmplified & Purified cDNA Volume** | **28 µl** | **28 µl** |
| Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* $H_2O$ | **192 µl** | **82 µl** |
| **Diluted PreAmplified & Purified cDNA Volume** | **220 µl** | **110 µl** |
| **cDNA volume for each qPCR reaction** | **2 µl** | **1 µl** |

2) Follow the SignArrays 94 or 384 Handbooks to perform your qPCR analyses.

## III. Additional Informations

For further information, please contact AnyGenes® technical support via the following email address : technical@anygenes.com

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