

## Globin Reduction PNA Kit

Globin Reduction PNA kit a novel, non-enzymatic technology that removes majority of alpha and beta globin mRNA from total RNA preparations derived from whole blood.

PNA (peptide nucleic acid) is a nucleic acid analog with has higher affinity and specificity than DNA or RNA. Because it cannot be recognized as a primer by polymerases, PNA can bind and block amplification of target nucleic acid.

PNA oligomers in Globin Reduction PNA Kit can specifically and efficiently block amplification of globin mRNA during the process of reverse transcription, resulting in specific PCR amplification of the target non-globin mRNA for your analysis.

Globin reduction PNA is composed of 4 PNA oligos.

PNA1: k-TAA CGG TAT TTG GAG-k

PNA2: k-GTA GTT GGA CTT AGG-k

PNA3: k-GCC CTT CAT AAT ATC-k

PNA4: k-ATC CAG ATG CTC AAG-k

These sequences are specific to human adult globin mRNA transcripts.

### PROTOCOL

| Tube | PNA sequence           | Dissolve volume (ul) | Stock concentration (uM) | Final working concentration (uM) | Required for 5ug RNA (ul) |
|------|------------------------|----------------------|--------------------------|----------------------------------|---------------------------|
| PNA1 | TAA CGG TAT TTG<br>GAG | 60                   | 50                       | 7.2                              | 0.144                     |
| PNA2 | GTA GTT GGA CTT<br>AGG | 60                   | 50                       | 12                               | 0.24                      |
| PNA3 | GCC CTT CAT AAT<br>ATC | 60                   | 50                       | 12                               | 0.24                      |
| PNA4 | ATC CAG ATG CTC<br>AAG | 60                   | 50                       | 12                               | 0.24                      |

1. Dissolve each globin reduction PNA with 60 ul of water to achieve 50 uM stock solution. PNA stock is stored frozen however working solutions are kept in room temperature.
2. Prepare PNA mix by adding 1.44 ul of PNA1, 2.4 ul of PNA 2~4, and 1.36 ul of water to make total of 10 ul (sufficient for 10 reaction, 5 ug RNA each).
3. Mix 5 ug of RNA (denatured and chilled on ice), oligo dT primer (50 uM) 1 ul, 1 ul of PNA mix in total of 10 ul of reaction volume. It is recommended to include a control reaction without PNA and without oligo dT primer.
4. Place the tube in PCR machine preheated to 70 oC and incubate for 10 min at 70 oC.
5. Put the mixture in ice. Continue to cDNA synthesis protocol.

## REFERENCES

1. Vartanian K. et al., *Gene expression profiling of whole blood: comparison of target preparation methods for accurate and reproducible microarray analysis*. **BMC Genomics** (2009)10:2.