WHAT IS CLAIMED IS:

1. A method of amplifying a nucleic acid molecule, the method comprising:

(a) providing the nucleic acid in a composition sufficient for nucleic acid amplification, the composition comprising:

(i) one or more oligonucleotide primers;

(ii) a polymerase; and

(iii) a sufficient amount of an agent to improve the specificity of nucleic acid amplification, wherein the agent comprises free arginine, spermidine, or spermine, or a salt thereof; and

(b) incubating the mixture under conditions sufficient for amplifying the nucleic acid, thereby amplifying the nucleic acid.

2. The method of claim 1, wherein the polymerase is conjugated to a DNA binding domain.

3. The method of claim 2, wherein the DNA binding domain is a Sso7 domain.

4. The method of claim 1, wherein the agent is present in an amount sufficient to increase by at least 10% the relative yield of a specific amplification product as compared to a non-specific amplification product.

5. The method of claim 1, wherein the agent is free arginine or a salt thereof.

6. The method of claim 5, wherein the concentration of the free arginine or the free arginine salt is from about 1 mM to about 500 mM.

7. The method of claim 1, wherein the polymerase substantially lacks a 3'-5' exonuclease activity.

8. The method of claim 1, wherein the polymerase has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

9. The method of claim 3, wherein the Sso7 domain has at least 75% amino acid sequence identity to SEQ ID NO:3.

10. The method of claim 1, wherein the composition comprises a buffer (when measured at a concentration of 0.1 M) that has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

11. The method of claim 10, wherein the buffer is selected from the group consisting of HEPES, ACES, PIPES, MOPSO, BES, MOPS, TES, TAPSO, POPSO, BICINE, TAPS, and AMPSO.

12. A method of amplifying a nucleic acid, the method comprising:

(a) providing the nucleic acid with a composition sufficient for nucleic acid amplification, the composition comprising:

(i) one or more oligonucleotide primers; and

(ii) a polymerase, wherein the polymerase is present at a concentration of at least 20 units/reaction; and

(b) incubating the nucleic acid in the composition under conditions sufficient for amplifying the nucleic acid, thereby amplifying the nucleic acid.

13. The method of claim 12, wherein the polymerase is conjugated to a DNA binding domain.

14. The method of claim 13, wherein the DNA binding domain is a Sso7 domain.

15. The method of claim 12, wherein the composition further comprises free arginine or a salt thereof.

16. The method of claim 15, wherein the concentration of the free arginine or the free arginine salt is from about 1 mM to about 500 mM.

17. The method of claim 12, wherein the polymerase substantially lacks a 3'-5' exonuclease activity.

18. The method of claim 12, wherein the polymerase has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

19. The method of claim 14, wherein the Sso7 domain has at least 75% amino acid sequence identity to SEQ ID NO:2.

20. The method of claim 12, wherein the composition comprises a buffer (when measured at a concentration of 0.1 M) that has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

21. The method of claim 20, wherein the buffer is selected from the group consisting of HEPES, ACES, PIPES, MOPSO, BES, MOPS, TES, TAPSO, POPSO, BICINE, TAPS, and AMPSO.

22. A reaction mixture comprising:

a polymerase; and

a sufficient amount of an agent to improve the specificity of nucleic acid amplification, wherein the agent comprises free arginine, spermidine, or spermine, or a salt thereof.

23. The reaction mixture of claim 22, wherein the polymerase is conjugated to a DNA binding domain.

24. The reaction mixture of claim 23, wherein the DNA binding domain is a Sso7 domain.

25. The reaction mixture of claim 22, wherein the agent is present in an amount sufficient to increase by at least 10% the relative yield of a specific amplification product as compared to a non-specific amplification product.

26. The reaction mixture of claim 22, wherein the agent is free arginine or a salt thereof.

27. The reaction mixture of claim 26, wherein the concentration of the free arginine or the free arginine salt is from about 1 mM to about 500 mM.

28. The reaction mixture of claim 22, wherein the polymerase substantially lacks a 3'-5' exonuclease activity.

29. The reaction mixture of claim 22, wherein the polymerase has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

30. The reaction mixture of claim 24, wherein the Sso7 domain has at least 75% amino acid sequence identity to SEQ ID NO:2.

31. The reaction mixture of claim 22, further comprising one or more oligonucleotide primers.

32. The reaction mixture of claim 22, further comprising at least one member selected from the group consisting of a buffer, nucleotide triphosphates, a salt, a stabilizer, a double stranded DNA binding dye, and nuclease-free water.

33. The method of claim 22, wherein the reaction mixture comprises a buffer (when measured at a concentration of 0.1 M) that has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

34. The method of claim 33, wherein the buffer is selected from the group consisting of HEPES, ACES, PIPES, MOPSO, BES, MOPS, TES, TAPSO, POPSO, BICINE, TAPS, and AMPSO.

35. A kit comprising:

a polymerase; and

a sufficient amount of an agent to improve the specificity of nucleic acid amplification, wherein the agent comprises free arginine, spermidine, or spermine, or a salt thereof.

36. The kit of claim 35, wherein the polymerase is conjugated to a DNA binding domain.

37. The kit of claim 36, wherein the DNA binding domain is a Sso7 domain.

38. The kit of claim 35, wherein the agent is present in an amount sufficient to increase by at least 10% the relative yield of a specific amplification product as compared to a non-specific amplification product.

39. The kit of claim 35, wherein the agent is arginine.

40. The kit of claim 39, wherein the concentration of free arginine is from about 1 mM to about 500 mM.

41. The kit of claim 35, wherein the polymerase substantially lacks a 3'-5' exonuclease activity.

42. The kit of claim 35, wherein the polymerase has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

43. The kit of claim 37, wherein the Sso7 domain has at least 75% amino acid sequence identity to SEQ ID NO:2.

44. The kit of claim 35, further comprising one or more oligonucleotide primers.

45. The kit of claim 35, further comprising at least one member selected from the group consisting of a buffer, nucleotide triphosphates, a salt, a stabilizer, a double stranded DNA binding dye, and nuclease-free water.

46. The kit of claim 35, wherein the polymerase and the agent are in a solution comprising a buffer, wherein the buffer (when measured at a concentration of 0.1 M) has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

47. The method of claim 46, wherein the buffer is selected from the group consisting of HEPES, ACES, PIPES, MOPSO, BES, MOPS, TES, TAPSO, POPSO, BICINE, TAPS, and AMPSO.

48. A reaction mixture for signal normalization in a real-time polymerase chain reaction (PCR) amplification of a target nucleic acid wherein the mixture is compatible for use in both (a) a real-time PCR amplification system employing a high passive reference dye concentration for the normalization and (b) a real-time PCR amplification system employing a low passive reference dye concentration for the normalization, wherein the mixture comprises:

a plurality of passive reference dyes that produces fluorescent signals independent of the amplification reactions; and

a buffer, wherein the buffer (when measured at a concentration of 0.1 M) has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

49. The mixture of claim 48,wherein the buffer is selected from the group consisting of HEPES, ACES, PIPES, MOPSO, BES, MOPS, TES, TAPSO, POPSO, BICINE, TAPS, and AMPSO.

50. The mixture of claim 48, further comprising a sufficient amount of an agent to improve the specificity of nucleic acid amplification, wherein the agent comprises free arginine, spermidine, or spermine, or a salt thereof.

51. The mixture of claim 48, the mixture comprising

a first passive reference dye having a Stokes-shift, wherein the first passive reference dye is at a concentration sufficient for use in low concentration passive reference dye normalization, wherein the first passive reference dye has a first passive reference dye excitation wavelength maximum and a first passive reference dye emission wavelength maximum; and

a second passive reference dye having a Stokes-shift that is greater than the Stokes-shift of the first passive reference dye, wherein the second passive reference dye has an emission wavelength maximum approximately the same as the first passive reference dye emission wavelength maximum, and an excitation wavelength maximum significantly different than the first passive reference dye excitation wavelength maximum.

52. The mixture of claim 51, wherein the second passive reference dye has a Stokes-shift of at least about 60 nm.

53. The mixture of claim 51, wherein the first passive reference dye comprises 5- and/or 6-carboxy-X-rhodamine, or an analog thereof.

54. The mixture of claim 51, wherein the second passive reference dye has a excitation wavelength maximum of 550 nm or less.

55. The mixture of claim 51, wherein the concentration of the 5- and/or 6-carboxy-X-rhodamine dye is less than 100 nM.

56. The mixture of claims 51, further comprising one or more of an oligonucleotide primer, one or more deoxynucleoside triphosphates; a buffer, an intercalating dye, a reverse transcriptase, and a polymerase.

57. The mixture of claim 56, comprising DNA polymerase.

58. The mixture of claim 57, wherein the polymerase is complexed with an antibody.

59. The mixture of claim 57, wherein the polymerase is chemically inactivated but is activated by heating.

60. The mixture of claim 51, wherein said second passive dye is a fluorescent dot.

61. The mixture of claim 51, wherein the second passive reference dye is conjugated to a moiety.

62. A method of performing a real-time quantitative polymerase chain reaction, the method comprising,

performing a polymerase chain reaction (PCR) with the mixture of claim 51, wherein the mixture further comprises a biological sample suspected of comprising a target nucleic acid.

63. A method of making the reaction mixture of claim 51, the method comprising mixing the plurality of passive reference dyes, thereby generating the reaction mixture.

64. A kit for performing a real-time quantitative polymerase chain reaction, the kit comprising:

a first passive reference dye having a Stokes-shift, wherein the first passive dye has a first passive reference dye excitation wavelength maximum and a first passive reference dye emission wavelength maximum;

a second passive reference dye having a Stokes-shift that is greater than the Stokes-shift of the first passive reference dye, wherein the second passive reference dye has an emission wavelength maximum approximately the same as the first passive reference dye emission wavelength maximum, and an excitation wavelength maximum significantly different than the first passive reference dye excitation wavelength maximum and

a buffer, wherein the buffer (when measured at a concentration of 0.1 M) has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

65. The kit of claim 64,wherein the buffer is selected from the group consisting of HEPES, ACES, PIPES, MOPSO, BES, MOPS, TES, TAPSO, POPSO, BICINE, TAPS, and AMPSO.

66. The kit of claim 64, further comprising a sufficient amount of an agent to improve the specificity of nucleic acid amplification, wherein the agent comprises free arginine, spermidine, or spermine, or a salt thereof.

67. The kit of claim 64, wherein the second passive reference dye has a Stokes-shift of at least about 60 nm.

68. The kit of claim 64, wherein the first passive reference dye comprises 5- and/or 6-carboxy-X-rhodamine, or an analog thereof.

69. The kit of claim 64, wherein the first passive reference dye comprises a 5- and/or 6-carboxy-X-rhodamine dye or an analog thereof, and
the second passive reference dye is a fluorescent dye having a Stokes-shift of at least 60 nm, wherein the second passive reference dye has an emission wavelength maximum of about 620 nm.

70. The kit of claim 64, the mixture comprising
a 5- and/or 6-carboxy-X-rhodamine dye; and
a second passive reference dye having a Stokes-shift of at least about 60 nm, wherein the second passive reference dye has an emission wavelength maximum of about 590 nm.

71. The kit of claim 64, further comprising one or more of: one or more deoxynucleoside triphosphates; one or more of an oligonucleotide primer, one or more deoxynucleoside triphosphates; a buffer, an intercalating dye, a reverse transcriptase, and a DNA polymerase.

72. The kit of claim 64, wherein the first passive reference dye and the second passive reference dye are contained in different vessels in the kit.

73. The kit of claim 64, wherein the first passive reference dye and the second passive reference dye are contained in the same vessel in the kit.

Claims

1. A method of amplifying a nucleic acid molecule, the method comprising:

(a) providing the nucleic acid in a composition sufficient for nucleic acid amplification, the composition comprising:

(i) one or more oligonucleotide primers;

(ii) a polymerase; and

(iii) a sufficient amount of an agent to improve the specificity of nucleic acid amplification, wherein the agent comprises free arginine, or a salt thereof, wherein the concentration of the free arginine or the salt thereof is from about 1 mM to about 25 mM; and

(b) incubating the mixture under conditions sufficient for amplifying the nucleic acid, thereby amplifying the nucleic acid.

2. The method of claim 1, wherein the polymerase is conjugated to a DNA binding domain.

3. The method of claim 2, wherein the DNA binding domain is a Sso7 domain.

4. The method of claim 1, wherein the agent is present in an amount sufficient to increase by at least 10% the relative yield of a specific amplification product as compared to a non-specific amplification product.

5. The method of claim 1, wherein the polymerase substantially lacks a 3'-5' exonuclease activity.

6. The method of claim 1, wherein the polymerase has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

7. The method of claim 3, wherein the Sso7 domain has at least 75% amino acid sequence identity to SEQ ID NO:3.

8. The method of claim 1, wherein the composition comprises a buffer (when measured at a concentration of 0.1 M) that has a change of no more than 0.027 pH units per degree C when between 20° and 37° C.

9. The method of claim 8, wherein the buffer is POPSO.

10. The method of claim 2, wherein the polymerase is at a concentration of at least 20 U/ml.