

What is claimed is:

1. A test composition suitable for determining the presence of methylated CpG sequences of the genes in samples, that is, extracting
 5 gDNA from samples and subjecting the extracted DNA to appropriate pre-treatments and chemical reactions before using the test composition to examine the presence of gene methylation, said test composition comprising:

(1) a gene-specific primer mixture for target genes is consisting of
 10 at least one forward primer and one reverse primer, the sequences of said primers are selected from the nucleotide sequences is one or more sequences containing at least 10 continuous nucleotides identical to the sequences indicated in SEQ ID No: 1-70;

wherein the forward and reverse primer of paired box protein 1
 15 (PAX1) are one of SEQ ID No. 1-25,

wherein the forward and reverse primer of zinc finger protein 582 (ZNF582) are one of SEQ ID No. 26-40,

wherein the forward and reverse primer of sex determining region Y-box 1 (SOX1) are one of SEQ ID No. 41-56,

20 wherein the forward and reverse primer of homeobox protein Nkx-6,1 (NKX6-1) are one of SEQ ID 57-70;

(2) a nucleic acid molecule test mixture comprising at least one forward primer and one reverse primer for internal control gene; wherein the internal control genes are selected from at least one or
 25 more of the following genes; Col2A, β -Globin, GAPDH, and β -actin;

(3) a master mixture for PCR, the main ingredients of said mixture include at least polymerase, dNTPs, and Magnesium,

wherein the test composition use the target genes in the samples are selected SOX1 could detect one of the following: oral cancer, head
5 and neck cancer, esophageal cancer, colorectal cancer, liver cancer, ovarian cancer, breast cancer, and tongue cancer;

or wherein the test composition use the target gene in the sample selected from PAX1 could detect one of the following: oral cancer, head and neck cancer, esophageal cancer, breast cancer, and tongue
10 cancer;

or wherein the test composition use the target gene in the sample selected from NKX6-1 could detect one of the following: oral cancer, head and neck cancer, esophageal cancer, colorectal cancer, liver cancer, breast cancer, and tongue cancer;

15 or wherein the test composition use the target gene in the sample selected from ZNF582 could detect one of the following: oral cancer, head and neck cancer, esophageal cancer, liver cancer, breast cancer and tongue cancer.

20 2. The test composition as recited in claim 1, wherein the features of the test composition are:

(1) the gene-specific primer mixture for target genes further comprises probes and the sequences of said probes are selected from

one or more or complementary sequences contain at least 10 continuous nucleotides identical to the sequences indicated in SEQ ID No:1-70;

wherein the forward and reverse primer of paired box protein 1 (PAX1) are one of SEQ ID No: 1-25,

wherein the forward and reverse primer of zinc finger protein 582 (ZNF582) are one of SEQ ID No. 26-40,

wherein the forward and reverse primer of sex determining region Y-box (SOX1) are one of SEQ ID No. 41-56,

wherein the forward and reverse primer of homeobox protein Nkx-6.1 (NKX6-1) are one of SEQ ID No. 57-70;

(2) the nucleic acid molecule test mixture for internal control genes further comprises the probes that are capable of detection of amplified PCR products of the internal control genes.

15

3. The test mixture as recited in claim 1, wherein the nucleic acid molecule test mixture for internal control genes contains forward primers and reverse primers and the sequences of said primers are selected from the nucleotide sequences, or is one or more sequences containing at least 10 continuous nucleotides identical to the sequences indicated in SEQ ID No: 71-80.

4. The test composition as recited in claim 2, wherein the nucleic acid molecule test mixture for internal control genes further comprises probes, wherein the sequences of the forward primers and reverse primers for the internal control genes are selected from the nucleotide

25

sequences, or is one or more or complementary sequences containing at least 10 continuous nucleotides identical to the sequences indicated in SEQ ID No: 71-80,

wherein the forward and reverse primer of PAX1 are one of
5 SEQ ID No. 1-25,

wherein the forward and reverse primer of ZNF582 are one of
SEQ ID No. 26-40,

wherein the forward and reverse primer of SOX1 are one of
SEQ ID No. 41-56,

10 wherein the forward and reverse primer of NKX6-1 are one of
SEQ ID No. 57-70; and

wherein the primers of internal control gene are Col2A, β -
Globin, GAPDH, or β -actin.

15 5. The test composition as recited in claim 1, wherein the sequences of
the forward primers and reverse primers for the target genes are
selected from the one or more of the nucleotide sequences indicated in
SEQ ID No: 1-70.

20 6. The target genes as recited in claim 2, wherein the sequences of the
forward primers and reverse primers are selected from the one or more
of the nucleotide sequences indicated in SEQ ID No: 1-70; the
sequences of the probes for the target genes are selected from one or
more or complementary sequences of the nucleotide sequences
25 indicated in SEQ ID No: 1-70.

7. The internal control genes as recited in claim 4, wherein the sequences of the forward primers and reverse primers are selected from one or more of the nucleotide sequences indicated in SEQ ID No: 71-80; the sequences of the probes for the internal control genes are
5 selected from one or more or complementary sequences of the nucleotide sequences indicated in SEQ ID No: 71-80.

8. The test composition as recited in claim 1, wherein the master mixture for PCR comprises a fluorescent substance that can identify
10 the amplified PCR products or a detectable substance that can react with double-stranded DNA and produce fluorescence.

9. The test composition as recited in claim 2, wherein the probes for the target genes and internal control genes are labeled with fluorescent
15 dyes, said fluorescent labels are selected from one of the following fluorescent dyes: FAM, HEX, TET, TAMRA, Cy3, Cy5, Cy5.5, VIC, Red610, Yellow 555, Texas Red, Yakima Yellow, BHQ-1, BHQ-2, and BHQ-3.

20 10. The test composition as recited in claim 1, wherein the test composition is capable of detecting abnormal cell proliferation.

11. The test composition as recited in claim 10, wherein detection of abnormal cell proliferation includes precancerous lesions, tumor
25 detection, detection of tumor recurrence, prediction of the effect of cancer drug, or detection of cancer outcomes.

12. The test composition as recited in claim 2, wherein the test composition is capable of detecting abnormal cell proliferation.
- 5 13. The test composition as recited in claim 12, wherein detection of abnormal cell proliferation includes precancerous lesions, tumor detection, detection of tumor recurrence, prediction of the effect of cancer drug, or detection of cancer outcomes.
- 10 14. The test composition as recited in claim 1, wherein the primers for the target genes and internal control genes can be mixed together for preparation of the nucleic acid molecule test mixture in a single tube.
- 15 15. The test composition as recited in claim 2, wherein the primers and probes for the target genes and the internal control genes can be mixed together for preparation of the nucleic acid molecule test mixture in a single tube.
- 20 16. The test composition as recited in claim 1 or 2, wherein the identification methods for amplified PCR products include fluorescence, sequencing, microarrays, mass spectrometer, denaturing high-performance liquid chromatography (DHPLC), pyrosequencing, or immunoassay.