# Qubit® dsDNA HS Assay Kits 操作说明书

**【产品名称】**

通用名称：双链 DNA 超敏检测试剂盒（荧光法）

英文名称：Qubit® dsDNA HS Assay Kits

**【包装规格】**

100 份&500 份/盒

**【预期用途】**

Nanodrop 系列微量分光光度计采用目前最常见的紫外吸光法对待测样品的浓度及纯度进行检测，由于它会检测吸光度在 260nm 处所有物质的吸光值（如 DNA、RNA、降解核酸和游离核苷酸等其他杂质），因此读数并不是十分准确。而荧光计通过荧光染料与特定目标分子结合后，检测其荧光强度来测目标分子的浓度，因此其定量结果一般低于 A260nm 处的读数，但是更为准确。

另外，准确的 DNA 定量对于许多后续应用而言都至关重要。Nanodrop 系列全波长微量分光光度计无法精确测定 5ng/ul 以下的 DNA 浓度，然而，许多 DNA 样品又恰在这个范围之内。在检测双链 DNA 时，荧光计的检测极限可达 0.5pg/ul。此时，更为灵敏的荧光计似乎是个更好的选择。 荧光计和相应的定量试剂盒，能快速灵敏、精确测定 DNA 的浓度。

本试剂将被用于成本昂贵的下游实验:qPCR 、PCR 克隆、转染和新一代测序等精密测定的实验，精准定量范围 10 pg /μL 至 100ng/μL。

**【检验原理】**

本试剂盒利用 Picogreen DNA 染料能够特异性结合双链 DNA，而不与蛋白质、RNA、盐离子结合的原理，通过激发光照射，Qubit 检测仪收集发射光，特异的分析样品中双链 DNA 的实际含量，能够排除样品中蛋白质、RNA、盐离子等物质的干扰，从而对双链 DNA 进行精准的定量。

**【主要组成成份】**

| 试剂盒组成 | 保存 | Q2401（100 人份） | Q2402（500 人份） |
|---|---|---|---|
| Qubit Reagent | 2-8℃ | 100 μL/支×1 支 | 500 μL/支×1 支 |
| Qubit dsDNA HS Standard #1 | 2-8℃ | 1mL/瓶×1 支 | 5mL/瓶×1 瓶 |
| Qubit dsDNA HS Standard #2 | 2-8℃ | 1mL/瓶×1 支 | 5mL/瓶×1 瓶 |
| Qubit dsDNA HS Buffer | 2-8℃ | 30mL /瓶×1 瓶 | 120mL /瓶×1 瓶 |
| 说明书 | 常温 | 1 份 | 1 份 |

备注：

① 不同批次的试剂不可混合使用。

**【储存条件及有效期】**

2-8℃保存；避免反复冻融；长期储存请放-20℃，有效期 24 个月。

**【适用仪器】**

荧光仪（Qubit 2.0 ， Qubit 3.0 等荧光仪）

**【需要但试剂盒没有提供】**

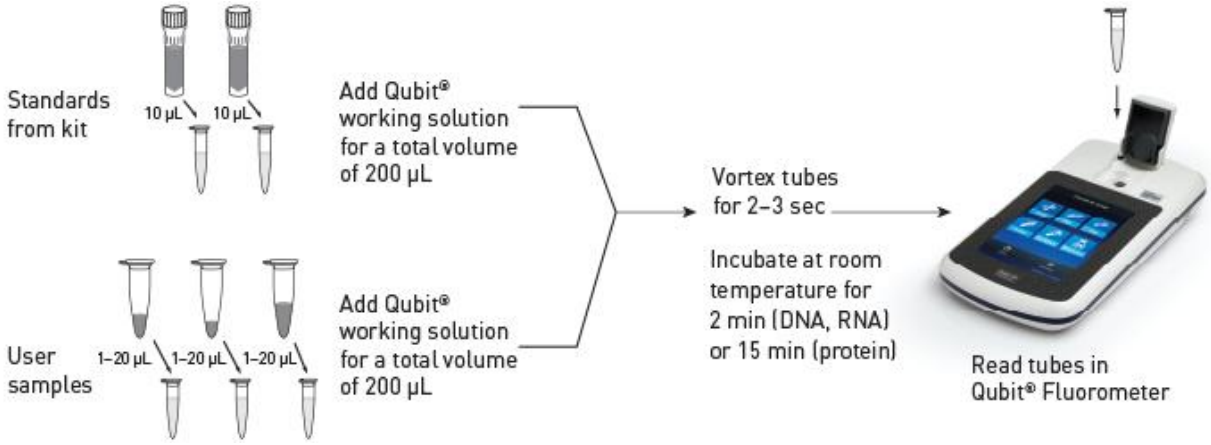
一次性无尘手套、 Qubit® assay tubes (500 tubes, Life Technologies, Cat. no. Q32856)或者 Axygen® PCR-05-C tubes （VWR, part no. 10011-830）

【检验方法】

## 1. 实验准备：

1.1 准备好检测所需要的 0.5ml 的薄壁离心管（n=待测样品数+2 standards）；
1.2 在薄壁离心管的管盖处进行标记样品的编号，切不可在管的侧壁进行标记。
1.3 Qubit® 工作液的准备：使用新的塑料管按照使用的量将 Qubit® Reagent 用 Qubit® dsDNA HS Buffer 按照 1:200 的比例进行稀释，例如：V（Qubit® 工作液）=（待测样品数+2 standards）×（1V $_{(Qubit®Reagent)}$ +199V $_{(Qubit® dsDNA HS Buffer)}$ ）涡旋混匀待用；
1.4 按 190μL 将 Qubit® 工作液分到标准品的 2 个离心管中，分别加入 10μL 标准品 Qubit dsDNA HS Standard #1 和 Qubit dsDNA HS Standard #2，涡旋混匀备用，注意不要出现气泡；
1.5 待测样品：根据实际需要的量，将 Qubit® 工作液进行分装，确保测量体积为 200μL。例如：若待测样品的体积为 5μL，则 Qubit® 工作液为 195μL 进行分装。
1.6 将待测样品加入对应的检测管中，确保终体积为 200μL；涡旋混匀，不要出现气泡。
1.7 将准备好的标准品和样品放置室温反应 3min，待测。

**操作流程图例：**



## 2. Qubit® 3.0 操作步骤

2.1 在 Qubit® 3.0 主屏幕操作页面，选择 **DNA**，然后选择检测类型 **dsDNA High Sensitivity**。显示“**Read standards**”，选择 **Read Standards** 进行检测。
2.2 将装有 Standard #1 管子插入样品槽中，关闭上盖， 按键 **Read standard**. 读取完成后(~3 seconds)， 移走 Standard #1。
2.3 将 装有 Standard #2 管子插入样品槽中，关闭上盖， 按键 **Read standard**. 读取完成后(~3 seconds)， 移走 Standard #2。
2.4 按键 **Run samples**。
2.5 在读取页面，选择相应的单位和体积；
2.5.1 从旋转轮面板上选择+或-按钮来选择添加到试管中的样品体积（从 1 - 20µl）；
2.5.2 从下拉菜单中选择测量样品浓度的浓度单位。
2.6 将装有**待测样品**的管子插入样品槽中，关闭上盖， 按键 **Read** ，读取完成后(~3 seconds), 移走样品，直至样品测完。

## 3. Qubit® 2.0 操作步骤

3.1 在 Qubit® 2.0 主屏幕操作页面，选择 **DNA**，然后选择检测类型 **dsDNA High Sensitivity**。显示“**Read standards**”，选择 **Read Standards** 进行检测。
3.2 将装有 Standard #1 的管子插入样品槽中，关闭上盖， 按键 **Read standard**. 读取完成后(~3 seconds)， 移走 Standard #1。
3.3 将 装有 Standard #2 管子插入样品槽中，关闭上盖， 按键 **Read standard**. 读取完成后(~3 seconds)， 移走 Standard #2。
3.4 按键 **Run samples**。
3.5 将装有**待测样品**的管子插入样品槽中，关闭上盖， 按键 **Read** ，读取完成后(~3 seconds), 移走样品，直至样品测完。
3.6 按照下面公式计算样品管中 DNA 的浓度。

$$\text{样品浓度} = \text{QF value} \times \frac{200}{X}$$

（ QF Value :Qubit® 2.0 直接测量得到样品的结果；X:加入的样品的体积）

## 【产品性能指标】

1. 最低检测限：10pg/µL
2. 核酸定量检测线性范围 10pg/µL-10ng/µL。
3. 特异性检测与 Mg2+、氯化钠、醋酸钠、醋酸铵、乙醇、苯酚、氯仿、SDS、RNA、蛋白质、Triton-X100、dNTPS、BSA 等干扰物没有交叉反应。

## 【注意事项】

1. 检测温度：所有检测必须在室温操作（ (22–28˚C)）；
2. 孵育时间：DNA 与工作液混合后，反应 3min，荧光信号在 3 个小时内都是稳定的。
3. 荧光校准：每次使用前，请按照批次使用校准，以确保实时检测数据的一致性。
4. 95%的客户使用过程中发现，使用体积为 1µl 的样品检测，误差偏差较大，不推荐体积＜2µl 的检测。
5. 室内若开空调的情况下，请远离空调的出风口检测，避免温度不稳定导致检测样品浓度的误差。

# Qubit® dsDNA HS Assay Kits

【English name】Qubit® dsDNA HS Assay Kits

【Packaging Specification】

100 preps& 500preps /package

【Intended Use】

The Nanodrop series of micro spectrophotometers use ultraviolet absorbance method to detect the concentration and purity of the sample. Since it detects the absorbance value of all substances at 260nm, the reading is not very accurate. While the fluorometer measures the concentration of the target molecule by detecting the fluorescence intensity after combining with a specific target molecule, so its quantitative result is generally lower than the reading at 260nm, but it is more accurate.

In addition, accurate DNA quantification is crucial for many subsequent applications. The Nanodrop series of full-spectrum micro spectrophotometers cannot accurately determine the DNA concentration below 5ng/ul, however, many DNA samples happen to be within this range. When detecting double-stranded DNA, the detection limit of the fluorometer can reach 0.5pg/ul. At this time, a more sensitive fluorometer seems to be a better choice. The fluorometer and the corresponding quantitative kit can quickly, sensitively and accurately determine the concentration of DNA. The reagent will be used in costly downstream experiments: precise determination experiments such as qPCR, PCR cloning, transfection and next-generation sequencing, with a precise quantification range of 10 pg /μL to 100ng/μL.

【Test Principle】

This kit utilizes the principle that the Picogreen DNA dye specifically binds to double-stranded DNA without binding to proteins, RNA, and salt ions. Through the excitation light irradiation, the Qubit detector collects the emitted light to specifically analyze the actual content of double-stranded DNA in the sample, which can eliminate the interference of substances such as proteins, RNA, and salt ions in the sample, thereby accurately quantifying the double-stranded DNA.

【Main Components】

| Contents | Storage | Q2401(100preps) | Q2402（500preps） |
|---|---|---|---|
| Qubit Reagent | 2-8℃ | 110 μL | 550 |
| Qubit dsDNA HS Standard #1 | 2-8℃ | 1mL | 5mL |
| Qubit dsDNA HS Standard #2 | 2-8℃ | 1mL | 5mL |
| Qubit dsDNA HS Buffer | 2-8℃ | 30mL | 120mL |
| Manual book | Room temp | 1 | 1 |

Remarks:

1. Reagents from different batches cannot be mixed.
2. For long-term storage, the dsDNA standards can be stored at ≤–20°C

【Storage Conditions and Expiry Date】

Store at 2-8℃; Avoid repeated freeze-thaw cycles; valid for 24 months.

【Applicable Instruments】

Fluorometer (Qubit 2.0, Qubit 3.0, Qubit 4.0 etc.)

【Required but not provided by the kit】

Disposable dust-free gloves, Qubit® assay tubes (500 tubes, Life Technologies, Cat. no. Q32856) or Axygen® PCR-05-C tubes (VWR, part no. 10011-830)

【Test Method】

1. Experimental preparation:

1.1 Prepare the required 0.5ml thin-walled centrifuge tubes (n = number of samples to be tested + 2 standards);

1.2 Mark the sample number on the cap of the thin-walled centrifuge tube, and do not mark on the side wall of the tube.

1.3 Preparation of Qubit® working fluid:

Use a new plastic tube to dilute the Qubit® Reagent with the Qubit® dsDNA HS Buffer according to the ratio of 1:200 according to the usage amount, for example: V (Qubit® working fluid) = (number of samples to be tested + 2 standards) × (1V (Qubit® Reagent) + 199V (Qubit® dsDNA HS Buffer)) and vortex to mix for later use;

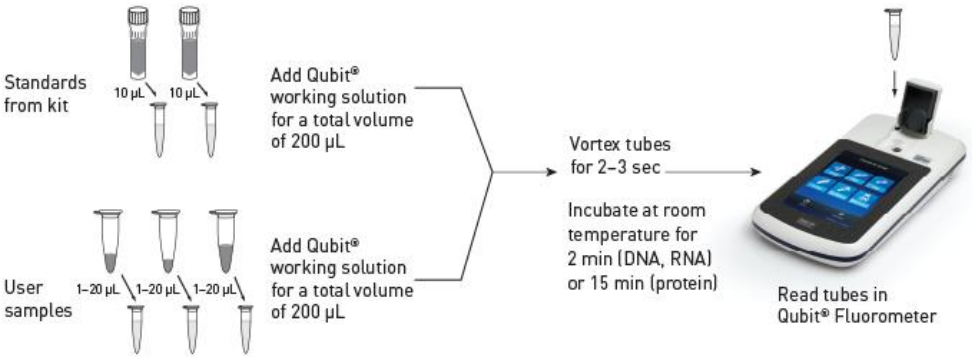
1.4 Dispense 190 μL of the Qubit® working fluid into 2 centrifuge tubes of the standard, and add 10 μL of the standard Qubit dsDNA HS Standard #1 and Qubit dsDNA HS Standard #2 respectively, and vortex to mix for later use, and be careful not to generate bubbles;

1.5 Samples to be tested: According to the actual required amount, distribute the Qubit® working fluid to ensure that the measurement volume is 200 μL. For example: if the volume of the sample to be tested is 5 μL, then 195 μL of the Qubit® working fluid is distributed.

1.6 Add the sample to be tested to the corresponding test tube to ensure that the final volume is 200 μL; vortex to mix without generating bubbles.

1.7 Place the prepared standards and samples at room temperature for 3 minutes for later testing.

Example of operation flow chart:




2. Qubit® 3.0 operation steps

2.1 On the Qubit® 3.0 main screen operation page, select DNA, and then select the detection type dsDNA High Sensitivity. Display "Read standards", select Read Standards for detection.

2.2 Insert the tube containing Standard #1 into the sample slot, close the upper cover, press the key Read standard. After reading is completed (~3 seconds), remove Standard #1.

2.3 Insert the tube containing Standard #2 into the sample slot, close the upper cover, press the key Read standard. After reading is completed (~3 seconds), remove Standard #2.

2.4 Press the Run samples key.

2.5 On the reading page, select the corresponding unit and volume;

2.5.1 Select the + or - button from the rotating wheel panel to select the sample volume added to the test tube (from 1 - 20 μl);

2.5.2 Select the concentration unit for measuring the sample concentration from the dropdown menu.

2.6 Insert the tube containing the sample to be tested into the sample slot, close the upper cover, press the key Read, after reading is completed (~3 seconds), remove the sample until all samples are tested.

3. Qubit® 2.0 operation steps

3.1 On the Qubit® 2.0 main screen operation page, select DNA, and then select the detection type dsDNA High Sensitivity. Display "Read standards", select Read Standards for detection.

3.2 Insert the tube containing Standard #1 into the sample slot, close the upper cover, press the key Read standard. After reading is completed (~3 seconds), remove Standard #1.

3.3 Insert the tube containing Standard #2 into the sample slot, close the upper cover, press the key Read standard. After reading is completed (~3 seconds), remove Standard #2.

3.4 Press the Run samples key.

3.5 Insert the tube containing the sample to be tested into the sample slot, close the upper cover, press the key Read, after reading is completed (~3 seconds), remove the sample until all samples are tested.

3.6 Calculate the concentration of DNA in the sample tube according to the following formula.

Sample concentration = QF value × (QF Value: The result directly measured by Qubit® 2.0 for the sample; X: the volume of the sample added)

【Product Performance Index】

1) Minimum detection limit: 10pg/μL
2) Nucleic acid quantitative detection linear range 10pg/μL-10ng/μL.
3) Specific detection has no cross-reaction with interfering substances such as Mg2+, sodium chloride, sodium acetate, ammonium acetate, ethanol, phenol, chloroform, RNA, proteins, Triton-X100, dNTPS, BSA, etc.

【Precautions】

① Detection temperature: All detections must be performed at room temperature ((22–28˚C));

② Incubation time: After DNA is mixed with the working fluid, the reaction lasts for 3 minutes, and the fluorescence signal is stable within 3 hours.

③ Fluorescence calibration: Please calibrate according to the batch before each use to ensure the consistency of real-time detection data.

④ 95% of customers found during use that the error deviation is relatively large when using a sample with a volume of 1 μl for detection, and a volume < 2 μl is not recommended for detection.

⑤ If the air conditioner is turned on indoors, please detect away from the air outlet of the air conditioner to avoid errors in the detection of the sample concentration due to unstable temperature.

【Contact Information】

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Production&Expiration Dates: See Label