Qubit™ 1X dsDNA HS Assay Kits

Catalog No. Q33230, Q33231

Pub. No. MAN0017455 **Rev.** A.0

Product information

The Qubit™ 1X dsDNA HS (High Sensitivity) Assay Kits make DNA quantitation easy and accurate. The kits include a ready-to-use assay buffer and DNA standards. To perform the assay, simply dilute your sample (any volume from 1–20 µL is acceptable) into the 1X working solution provided, then read the concentration using the Qubit™ Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (Figure 1, page 7) and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL, or 0.1 ng to 100 ng of DNA in the sample. The assay is performed at room temperature, and the signal is stable for 3 hours when the samples are protected from light. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (Table 2, page 8). In addition to the Qubit™ 1X dsDNA HS Assay Kits described here, we also offer other kits for assaying RNA, protein, and dsDNA at a higher concentration range (Table 3, page 9) as well as the Qubit™ dsDNA HS Assay - Lambda Standard (Cat. No. Q33233).

Note: This Qubit™ assay kit can be used with Qubit™ 2.0, 3, and 4 Fluorometer models. To use the assay with the Qubit™ 2.0 and 3 models, you will need to download and install the appropriate program file from **thermofisher.com/qubit**.

Table 1. Contents and storage

Material	Amount		Concentration	Storage*
	Q33230 (100 assays)	Q33231 (500 assays)		
Qubit™ 1X dsDNA HS Working Solution (Component A)	50 mL	250 mL	1X	• 2–8°C • Protect from light
Qubit™ 1X dsDNA HS Standard #1 (Component B)	1 mL	5 mL	0 ng/µL in TE buffer	2–8°C
Qubit™ 1X dsDNA HS Standard #2 (Component C)	1 mL	5 mL	10 ng/µL in TE buffer	
* When stored as directed, the kits are stable for at least 6 months from the date of receipt.				

Materials required but not provided

- Nuclease-free pipettors and tips
- Qubit™ assay tubes (Cat. No. Q32856)

Critical assay parameters

Assay temperature

The Qubit™ 1X dsDNA HS Assay delivers optimal performance when all solutions are at room temperature (18–28°C). Temperature fluctuations can influence the accuracy of the assay (Figure 2, page 7).

To minimize temperature fluctuations, insert all assay tubes into the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence; the Qubit™ Fluorometers can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading because this warms the solution and results in a different reading.

Incubation time

To allow the Qubit™ 1X dsDNA HS Assay to reach optimal fluorescence, incubate the tubes for 2 minutes after mixing the sample or the standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature when samples are protected from light.

Photostability of Qubit™ reagents

The Qubit™ reagents exhibit high photostability in the Qubit™ Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. However, if the assay tube remains in the Qubit™ Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (Figure 2, page 7). Note that the temperature inside the Qubit™ Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Qubit™ Fluorometer calibration

For each assay, you have the choice to run a new calibration or use the values from the previous calibration. When you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can decide how comfortable you are using the calibration data stored from the last time the instrument was calibrated. Additionally, remember that the fluorescence signal in the tubes containing standards and samples is stable for no longer than 3 hours. See Figure 3 (page 8) for an example of the calibration curve used to generate the quantification results.

Handling and disposal

No data are currently available that address the mutagenicity or toxicity of the Qubit™ 1X dsDNA HS Reagent (the dye in Component A). This reagent is known to bind nucleic acids. Treat the Qubit™ 1X dsDNA HS buffer with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Prepare standards and samples

This protocol assumes that you are preparing standards for calibrating the Qubit™ Fluorometer. If you plan to use the last calibration performed on the instrument (see "Qubit™ Fluorometer calibration", page 2), you need fewer tubes (step 1.1) and less working solution (step 1.3).

1.1 Set up the required number of 0.5-mL tubes for standards and samples. The Qubit™ 1X dsDNA HS Assay requires 2 standards.

Note: Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (Cat. No. Q32856)

1.2 Label the tube lids.

Note: Do not label the side of the tube as this could interfere with the sample read. Label the lid of each standard tube correctly. Calibration of the Qubit™ Fluorometer requires the standards to be inserted into the instrument in the right order.

1.3 Add 10 µL of each Qubit™ standard to the appropriate tube.

1.4 Add 1–20 µL of each user sample to the appropriate tube.

Note: If you are adding 1–2 µL of sample, use a P-2 pipette for best results.

1.5 Add the Qubit™ 1X dsDNA 1X buffer to each tube such that the final volume is 200 µL.

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180–199 µL. Ensure that you have sufficient Qubit™ working solution to accommodate all standards and samples.

Note: To avoid any cross-contamination, we recommend that you remove the total amount of working solution required for your samples and standards from the working solution bottle and then add the required volume to the appropriate tubes instead of pipetting directly from the bottle to each tube.

1.6 Mix each sample vigorously by vortexing for 3–5 seconds.

1.7 Allow all tubes to incubate at room temperature for 2 minutes, then proceed to "Read standards and samples". Follow the procedure appropriate for your instrument:

- Qubit™ 4 Fluorometer
- Qubit™ 3 Fluorometer
- Qubit™ 2.0 Fluorometer

Read standards and samples

Qubit™ 3 and Qubit™ 4 Fluorometers

2.1 On the **Home** screen of the Qubit™ 3 or the Qubit™ 4 Fluorometer, press **DNA**, then select **1X dsDNA HS** as the assay type. The "Read standards" screen is displayed. Press **Read Standards** to proceed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, skip to step 2.4. Otherwise, continue with step 2.2.

2.2 Insert the tube containing Standard #1 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete (~3 seconds), remove Standard #1.

2.3 Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read standard**. When the reading is complete, remove Standard #2.

The instrument displays the results on the Read standard screen. For information on interpreting the calibration results, refer to the *Qubit™ Fluorometer User Guide*, available for download at **thermofisher.com/qubit**.

2.4 Press **Run samples**.

2.5 On the assay screen, select the sample volume and units:

a. Press the **+** or **–** buttons on the wheel, or anywhere on the wheel itself, to select the **sample volume** added to the assay tube (from 1–20 µL).

b. From the **unit** dropdown menu, select the units for the output sample concentration.

2.6 Insert a sample tube into the sample chamber, close the lid, then press **Read tube**. When the reading is complete (~3 seconds), remove the sample tube.

The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration. For information on interpreting the sample results, refer to the *Qubit™ Fluorometer User Guide*.

2.7 Repeat step 2.6 until all samples have been read.

Qubit™ 2.0 Fluorometer

3.1 On the **Home** screen of the Qubit™ 2.0 Fluorometer, press **DNA**, then select **x dsDNA HS** as the assay type. The Standards screen is displayed.

Note: If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration. If you want to use the previous calibration, press **No** and skip to step 3.5. Otherwise, continue with step 3.2.

3.2 On the **Standards** screen, press **Yes** to read the standards.

3.3 Insert the tube containing Standard #1 into the sample chamber, close the lid, then press **Read**. When the reading is complete (~3 seconds), remove Standard #1.

3.4 Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read**. When the reading is complete, remove Standard #2.

When the calibration is complete, the instrument displays the Sample screen.

3.5 Insert a sample tube into the sample chamber, close the lid, then press **Read**. When the reading is complete (~3 seconds), remove the sample tube.

The instrument displays the results on the Sample screen. The value displayed corresponds to the concentration after your sample was diluted into the assay tube. To find the concentration of your original sample, record this value and perform the calculation yourself (see "Calculating the sample concentration") or let the instrument perform this calculation for you (see "Dilution Calculator").

3.6 Repeat step 3.5 until all samples have been read.

Calculate the sample concentration – Qubit™ 2.0 Fluorometer

Note: The Qubit™ 4 and 3 Fluorometers perform this calculation automatically.

The Qubit™ 2.0 Fluorometer gives values for the Qubit™ 1X dsDNA HS Assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \frac{200}{x}$$

where QF value = the value given by the Qubit™ 2.0 Fluorometer
x = the number of microliters of sample added to the assay tube

This equation generates a result with the same units as the value given by the Qubit™ 2.0 Fluorometer. For example, if the Qubit™ 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation is in ng/mL.

Dilution Calculator – Qubit™ 2.0 Fluorometer

The Dilution Calculator feature of the Qubit™ 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you added to the assay tube. To have the Qubit™ 2.0 Fluorometer perform this calculation for you, follow the instructions below.

4.1 After the sample measurement is complete, press **Calculate Stock Conc**. The Dilution Calculator screen is displayed.

4.2 Using the **volume** roller wheel, select the volume of your original sample that you added to the assay tube. When you stop scrolling, the Qubit™ 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

4.3 To change the units in which the original sample concentration is displayed:

a. Press **ng/mL**.

b. On the **unit selection** pop-up window, select a unit for your original sample concentration.

c. Touch anywhere on the screen to close the pop-up window. The Qubit™ 2.0 Fluorometer automatically converts the units to your selection.

Note: The unit button next to your sample concentration reflects the change in units. For example, if you changed the unit to pg/µL, the button displays pg/µL.

4.4 To save the data from your calculation to the Qubit™ 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement is saved in the *.csv file and tagged with a time and date stamp.

4.5 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or press **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit™ 2.0 Fluorometer saves the last values for the sample volume and units on the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit™ 1X dsDNA HS Assay

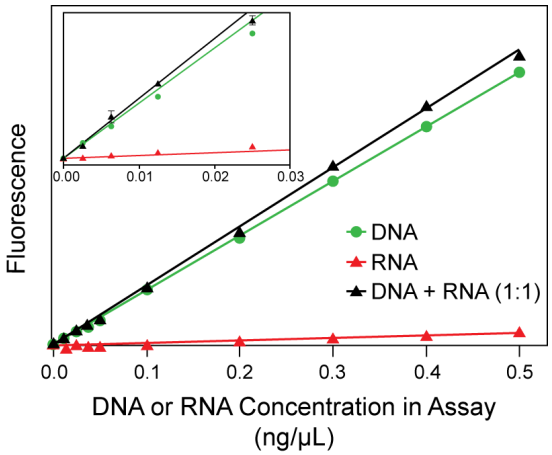

Figure 1. DNA selectivity and sensitivity of the Qubit™ 1X dsDNA HS Assay (Cat. Nos. Q33230, Q33231). Triplicate 10-µL samples of λ DNA (Cat. No. Q33233), *E. coli* rRNA, or a 1:1 Mixture of DNA and RNA were assayed with the Qubit™ dsDNA HS Assay. Fluorescence was measured and plotted versus the concentration of the RNA or DNA sample alone, or versus the mass of the DNA component in the 1:1 Mixture. The variation (CV) of replicate DNA determinations was ≤2%. The inset is an expanded view of the low range of the assay showing the extreme sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

Effect of temperature on the Qubit™ 1X dsDNA HS Assay

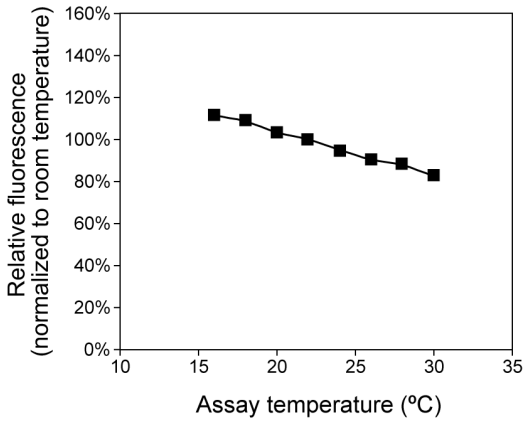

Figure 2. Plot of fluorescence vs. temperature for the Qubit™ 1X dsDNA HS Assay. The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit™ Fluorometer calculates concentration

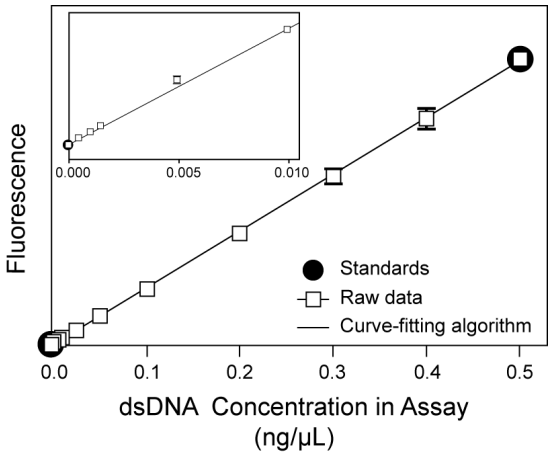

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit™ 1X dsDNA HS Assay. The Qubit™ Fluorometer generates concentration data based on the relationship between the two standards used in the calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ 1X dsDNA HS Assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation. The application note "Comparison of Quant-iT™ and Qubit™ quantitation assays for accuracy and precision" (available at **thermofisher.com/qubit**) describes in greater detail how the optimized Qubit™ algorithm provides improved accuracy and precision at the low end of the concentration range (<10 ng/mL) over traditional 8-point standard curve and linear regression.

Contaminants tolerated by the Qubit™ 1X dsDNA HS Assay

Note: While the contaminant tolerances of the Qubit™ 1X dsDNA HS assay and the Qubit™ dsDNA HS assay are largely similar, they are not identical.

Table 2. Effect of contaminants in the Qubit™ 1X dsDNA HS Assay*

Contaminant	Final concentration in the assay	Concentration in 10-µL sample	Concentration in 1-µL sample	Result
Sodium chloride	50 mM	1 M	10 M	OK
Magnesium chloride	1 mM	20 mM	200 mM	OK
Sodium acetate	30 mM	600 mM	6 M	OK
Ammonium acetate	25 mM	500 mM	NA	OK
Sodium azide	1 mM	20 mM	200 mM	OK
Ethanol	1%	20 %	NA**	OK
Phenol	0.1%	2%	20%	OK
Chloroform†	1%	20 %	NA**	OK
SDS	0.002%	0.04%	0.4%	OK
BSA	1X‡	1X‡	1X‡	OK
IgG	1X‡	1X‡	1X‡	OK
ssDNA	1X‡	1X‡	1X‡	OK
RNA	1X‡	1X‡	1X‡	OK
dNTPs§	1X‡	1X‡	1X‡	OK

* DNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 10 µL or 1 µL sample volumes are also listed. In all cases, results are given as OK, usually less than 10% perturbation. For best results, add the same amount of contaminant to the standard samples.

** User sample would require greater than 100% of listed contaminant.

† Immiscible.

‡ 1X indicates a concentration equal to the concentration of dsDNA.

§ A mixture of dATP, dCTP, dGTP, and dTTP.

NA: Not available.

Qubit™ assay kits compatible with the Qubit™ Fluorometer

A number of fluorescence-based quantification kits are available for use with the Qubit™ Fluorometer. Use Table 3 to choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ assay kits for use with the Qubit™ Fluorometer

Product	Cat. No.	No, of assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• Core range (high confidence): 0.01 µg/mL to 5 µg/mL† • Extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • Useful for quantitation of genomic and miniprep DNA samples • Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851 Q33230	100	dsDNA	• Core range (high confidence): 1 ng/mL to 500 ng/mL† • Extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • Useful for quantitation of PCR products, viral DNA, and samples for subcloning • Accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
	Q32854 Q33231	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• Core range (high confidence): 5 ng/mL to 1000 ng/mL† • Extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1000 ng/mL to 1200 ng/mL† • Useful for quantitation of oligos, primers, denatured DNA, PCR products • Accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA HS Assay Kit	Q32852	100	RNA	• Core range (high confidence): 25 ng/mL to 500 ng/mL† • Extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1000 ng/mL† • Useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• Core range (high confidence): 0.1 µg/mL to 5 µg/mL† • Extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5 µg/mL to 6 µg/mL† • Useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • Accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
	Q10211	500		
Qubit™ microRNA Assay Kit	Q32880	100	RNA	• Core range (high confidence): 5 ng/mL to 500 ng/mL† • Extended ranges (moderate confidence): 2.5 ng/mL to 5 ng/mL and 500 ng/mL to 750 ng/mL† • Useful for quantification of samples for qRT-PCR and sequencing applications • Accurate in the presence of rRNA, large mRNA (>1000 bp), salts, solvents, proteins, and free nucleotides
	Q32881	500		
Qubit™ Protein Assay Kit	Q33211	100	Protein	• Core range (high confidence): 1.25 µg/mL to 25 µg/mL† • Extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • Little protein-to-protein difference in signal • Accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • Signal is stable for 3 hours
	Q33212	500		
Qubit™ RNA IQ Assay Kit	Q33221	75	RNA integrity and quality	• Although small in size, the tertiary structure of 5s and tRNA will bind the large RNA dye • Accurate in the presence of salts, protein, solvents and RNA stabilization reagents • Signal is stable for 1 hour • For use with the Qubit™ 4 Fluorometer; the assay does not work on the original Qubit™, Qubit™ 2.0, or Qubit™ 3 Fluorometers
	Q33222	275		

*Based on an assay volume of 200 µL.

†Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Ordering information

Cat. No.	Product name	Unit size
Q33230	Qubit™ 1X dsDNA HS Assay Kit, 100 assays	1 kit
Q33231	Qubit™ 1X dsDNA HS Assay Kit, 500 assays	1 kit
Related products		
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit™ Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32852	Qubit™ RNA HS Assay Kit, 100 assays *5–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32855	Qubit™ RNA HS Assay Kit, 500 assays *5–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q33221	Qubit™ RNA IQ Assay Kit, 75 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Q33222	Qubit™ RNA IQ Assay Kit, 275 assays *for use with the Qubit™ 4 Fluorometer*	1 kit
Q32880	Qubit™ microRNA Assay Kit, 100 assays *1–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q32881	Qubit™ microRNA Assay Kit, 500 assays *1–100 ng* *for use with the Qubit™ Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit™ Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit™ Fluorometer*	1 kit
Q33233	Qubit™ 1X dsDNA HS Assay - Lambda Standard	5 mL
Q32856	Qubit™ assay tubes *set of 500*	1 set

Documentation and support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision	Date	Description
A.0	08 November 2017	New user guide

Manufacturer: Life Technologies Corporation | 29851 Willow Creek Road | Eugene, OR 97402

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